FROM SCIENCE TO SCIENCE COMMUNICATION:

UNDERSTANDING EFFECTS AND PERCEPTIONS OF PHARMACEUTICALS

IN THE ENVIRONMENT

A THESIS

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BRITTANY MAULE

DR. MELODY BERNOT – ADVISOR

BALL STATE UNIVERSITY

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Table of Contents:

Project Overview:

An emerging issue of environmental concern is the presence of trace organic contaminants (TOCs) in aquatic systems. These pollutants include a wide variety of personal care products and pharmaceuticals that are introduced into the environment through human use. Specifically, products mainly enter through the wastewater stream and are not completely broken down through wastewater treatment practices. Effects on aquatic organisms have been measured across trophic levels on multiple endpoints such as reproduction, mortality, and biodiversity; however, effects on primary producers are less understood. With the potential to drive ecosystem function, understanding effects on algae- a key primary producer in aquatic systems- are essential to understanding whole ecosystem effects. Additionally, because of the pathways in which these compounds enter the environment, it is important to understand how to affect individual behavior to mitigate environmental impacts. Thus, the effects of five TOCs on algae function and community structure were assessed (Chapter 2) in addition to what mode of information delivery would be best to result in positive behavior choices associated with this issue (Chapter 1).

Chapter #1: Does the mode matter? Attitudes on environmental issues and behaviors in response to different modes of information delivery

Introduction:

Since 1957, surveys in the United States have consistently reported that Americans have low science literacy (Withey, 1959; Durant et al. 1989; Gregory and Miller, 1998). Specifically, in 2006, the United States ranked in the bottom 50% of science literacy globally (Organization for Economic Cooperation and Development, 2006). Understanding scientific issues is critical to personal, political, and global decisions. Thus, the need for effective science communication is paramount. The "deficit model" highlights lack of science literacy as the reason for decreased engagement with, and understanding of, scientific issues (Bodmer, 1985). This model contests that with more information, scientific knowledge as well as attitudes towards science will increase, leading to a more engaged public. Other theories indicate exposure to more information does not consistently translate into changes in behavior or attitudes, particularly if scientific information does not reinforce individual beliefs (Lord et al. 1979; Ditto and Lopez, 1992). Additionally, it is argued that other relationships to, and knowledge about, science outside the subject matter being addressed may play a greater role in determining attitudes and engagement (Wynne, 1992; Jasanoff, 2000; Sturgis and Allum, 2004). Attempts to understand the role these theories play in effective science communication have spurred much study and debate (Bauer et al. 2007; Nisbet and Mooney, 2007). It is clear there is a disconnect between the general public and scientific information which needs to remain at the forefront of science communication research. To effectively address this issue, research is needed to identify effective ways to engage audiences and transfer scientific information to change behaviors and attitudes (Nisbet and Scheufele, 2009).

Many individuals now seek information online, as use of technology continues to grow. Sixty percent of Americans use internet sources to seek out scientific information instead of print media (Science and Engineering Indicators, 2012). In addition, science concepts are delivered through videos on popular websites such as YouTube, which is ranked as the second most popular site both in the U.S. and globally (Alexa Internet Inc., 2015). Currently, students are also exposed to more technology in the classroom creating even more interaction with multimedia learning (Purcell et al. 2013). With an increasing amount of science information being sought online, and the wide variety in which consumers can obtain information (websites, videos, podcasts, etc.), understanding what mode of communication is most effective for transferring scientific information is vital to achieving change in literacy and behavior.

Opposed to the large body of literature on the benefits of active learning (e.g., Armbruster et al. 2009; Hoban et al. 2013), less is known on how the way information is presented affects behavior. Past research suggests auditory narration promotes better recall, relative to textual information, when viewing visual information simultaneously. This is due to differences in processing pathways that affect short-term memory (Penney, 1989; Levin and Divine-Hawkins, 1974). Because of these differing pathways, multimodality media can affect knowledge retention in learners (Mayer et al. 2001; Lowe, 2003; Oberfoell and Correia, 2016). For example, exposure to more information such as audio, visuals, text, and animations all at once can overstimulate and decrease the amount of information retained. Several studies have attempted to measure environmental attitudes on certain issues (Scott and Willits, 1994; Wray-Lake et al. 2010), and understand why these attitudes do not always translate into positive environmental behaviors (Courtenay-Hall and Rogers, 2002; Kennedy et al. 2009; Cham and

Lao, 2011). However, it is unclear whether changes in learning associated with differential modes of information exposure also translate to changes in behaviors and attitudes.

One example of potential actionable changes in behaviors all individuals can make is in the emerging issue of pharmaceuticals and personal care products (PPCPs) in the environment. This group of contaminants includes antimicrobials, sunscreens, insect repellents, fragrances, and prescription drugs, as well as stimulants such as caffeine and nicotine (Kolpin et al. 2002; Murray et al. 2010; Boxall et al. 2012; Rosi-Marshall and Royer, 2012). PPCPs are used by humans every day and are largely introduced through wastewater streams from human use and improper disposal (Boxall et al. 2012). The presence of these compounds in aquatic systems are well documented (Kolpin et al. 2002; Bernot et al. 2016) with potential adverse effects on aquatic ecosystems (Niemuth and Klaper, 2015; Shaw et al. 2015) and human health (Bolong et al. 2009). Individuals can minimize adverse effects through knowledgeable product consumption and proper disposal providing potential actionable change on an environmental issue.

In this study, we tested how different modes of communication affect understanding, perception and behaviors associated with environmental issues. We focused on transferring information regarding PPCPs in the environment, as it is an emerging issue with specific actionable changes individuals can make to reduce adverse environmental effects. Our target audience was middle school students in central Indiana, a group that has always had access to technology and information online (Prensky, 2001). It was hypothesized that exposure to different forms of information delivery would affect student information retention. Specifically, students exposed to a video, in which narration and visual images were used, would have increased retention of material relative to a pamphlet that presents only visual and textual information. We further hypothesized that the information delivery (hereafter referred to as “communication modes”) that increased retention would be more likely to alter behavior choices and student attitudes.

Methods:

Communication Modes

Three communication modes were developed to relay information on water quality to student participants. These communication modes included an informational website, pamphlet, and video. For each communication mode, design principles were followed to attempt to maximize the quality of design (Lidwell et al. 2010). All images, video, infographics, and text were either produced by the authors or cited within each mode. Text written or spoken was consistent across all modes to ensure participants were exposed to equal content with some small alterations in accordance with mode designs. Piktochart (Piktochart, 2017) and Adobe® Photoshop® (CC2015.x) an imaging editing software were used to create infographics and edit images in all modes.

Website: The website communication mode was developed using the free website building and hosting software, Weebly (Weebly, Inc. 2016). This software was chosen because of the user-friendly interface and design choices. The website consisted of five pages. The home page gave students a brief introduction as well as provided a link to the post survey. The four additional pages were used to divide the information into organized subheadings that were visible through the navigation bar: “Why do we care about water?”, “How does water move?”, “How does pollution affect water?”, and “How can we help?”

Pamphlet: The pamphlet communication mode was created using Microsoft Publisher 2016 (Microsoft, 2017). Microsoft Publisher was chosen because of its accessibility and previous experience using this software. The pamphlet consisted of two full 11”x8.5” pages printed on one two-sided piece of paper in landscape orientation. Headings in the pamphlet were similar to the website: “How Do Humans Influence Water Quality?”, “Freshwater Pollution: How Does It Get There?”, “How Does Pollution Affect Water?”, and “How Can We Help?” Pamphlets were printed in color and laminated to ensure all classes received the same pamphlets for the activity.

Video: Video content was filmed using the iPhone 5C and edited using HitFilm 4 Express, (FXHOME Limited, 2017), a free software program available for Windows. This software was chosen because of its accessibility and wide-range of tools available for editing video and images. The video contained time-lapse sequences, videos of waterways, organisms, and humans performing actions such as buying products and engaging in recreation activities. The topics covered in the video were similar to the two other modes. Topics in the video included the movement of water, its uses as an ecosystem service, effects of human pollution on freshwater, and ideas for how individuals could reduce pollutants. The video also used some of the still images found in both the pamphlet and website. Audio script was recorded using a Tascam DR-05 Linear PCM Digital Voice Recorder. Other audio files used in the video were labeled as royalty free music and attributed appropriately in the film. All audio files were also edited using HitFilm 4 Express.

Survey Instrument Development

Survey instrument questions were developed using a combination of multiple-choice, Likert scale, scenario, and short answer questions to gain a multi-faceted understanding of student attitude, learning, and behavior choices before and after the activity. Pre and post surveys were developed with similar questions to assess changes in response to information exposure. The pre survey consisted of a total of seven multiple-choice questions with three questions focused on assessing current habits in exposure to information, three scenario questions evaluating behavior choices, and one knowledge question. The pre survey also had two Likert scale questions (1-10) to quantify individual concern about water use and water quality. Three short answer questions regarding freshwater pollution were also in the pre survey. Lastly, four questions were modified from the New Ecological Paradigm (NEP) (Anderson, 2012) to gauge positive and negative associations towards environmental issues. The post survey was identical to the pre survey except that it did not contain the first two multiple-choice questions quantifying information exposure habits. The post survey also contained three more Likert scale questions (1-10) in which students were asked about how enjoyable and effective the activity was at fostering learning. In addition, the post survey included an open-ended suggestion prompt for ways the activity could be improved. A second post survey was conducted approximately five weeks after the activity to assess the effect of communication mode viewed on longer-term retention of knowledge. This second post survey included the same four questions assessing knowledge from the pre-survey (one multiple-choice and three short answer questions). The timeline for the second post survey was selected to accommodate school academic schedules and ensure students had approximately the same period of time between the initial post survey and second post survey.

Participant Recruitment

In accordance with IRB protocols (IRB # 876789-2), students were recruited through an initial contact to teachers and school administrator via email. Faculty and staff were not contacted more than one time. Once interest and willingness with educators was established, parental permission forms were distributed for student participation. On the day of the study, students were also given a child assent form to participate in the study. Only students having both parental permission and individual assent participated in study activities.

Student Population

Two hundred and ninety-one students participated in the study from three schools in Delaware County, Indiana: Highland Middle School (165), Yorktown Middle School (53), and Burris Laboratory School (73). All students were enrolled in eighth grade except for two classes from Burris Laboratory School that were sixth grade classes (49 of the 73 Burris students). Students from this demographic were chosen because required state educational standards for middle school students focused on the subject of this study (water quality and human impacts on the environment). The majority of participants in the target population were familiar with the communication modes presented based on pre survey responses: ~50% of student participants used video or website information sources to learn information at least weekly, and only ~8% of participants had never used these resources (Table 1). In addition, 191 students indicated they were likely to use websites to seek out information on environmental issues and 160 students also indicated they would use online videos for that purpose (*data not shown*).

Administration of Modes and Surveys

The survey instruments (pre and post survey) were administered to students using Google Forms. Students accessed these surveys via URL links. All student responses were anonymous and individual student responses to the pre and post survey were not paired. In a few instances where web access was inhibited (N = 3 students), paper instruments were provided. The website was also accessed via links. In contrast, the students who viewed the pamphlet were provided with the pamphlet as a handout. Viewing of all communication modes was limited to a maximum time of ten minutes, i.e., students browsing the website or reading the pamphlet had ten minutes to read and view the information. However, students were not required to use the full ten minutes and could stop viewing the communication mode at any time and continue to the post survey.

The video (approximately five minutes long) was viewed only one time. The video was played for each class on a projector to minimize technological issues on individual student devices.

Statistical Analysis

Data analysis included all student responses, with no separation based on grade or school. If a response to a question was missing or not fully completed (0.1%-12.2% depending on question) the response was excluded from analyses. As a result, the total number of responses varied for each question.

Knowledge Question: Student responses for the multiple-choice question: "What percentage of water on Earth is drinkable?" were counted and separated based on the communication mode viewed. A Chi-squared test was used to assess whether the distribution of student responses in the pre survey and post survey were different among the communication modes. All Chi-squared analyses were conducted using SigmaPlot Version 12.3.

Attitude Questions: Attitude questions included the four modified NEP statements, as well as the two questions addressing concern over water quality and water use. Average student responses in the pre and post survey for each communication mode were compared using a student's t-test with an α of 0.05 (R Core Team, 2016). Changes in the water use and water concern questions between the pre and post survey were then analyzed by pairing chronologically sorted student responses in each communication mode. Initial paired student responses were subtracted from the final responses to determine the change in student response for each mode. The changes in student response were then compared among the communication modes for each question using ANOVA with an α of 0.05 (R Core Team, 2016).

Scenario Questions: In each of the scenario questions, the proportion of responses was compared between the pre and post survey using a Chi-squared test as described above. When possible options were selected less than 10% of the total responses, the option was eliminated for analyses to meet Chi-square assumptions. The majority of instances where options were eliminated for statistical analyses were related to the student response option of “Other” where they could provide alternative answers. In addition, two answer options (“Take the medication to city hall” and “Take the medication to a pharmacy”) were combined and termed: “Take the medication to a drop-off location” for statistical analyses.

Student Perception Questions: Student responses to the Likert Scale questions in the post survey regarding perception of the activity were compared using an ANOVA with an α of 0.05 (R Core Team, 2016). In all ANOVA analyses, when significant differences were identified, they were followed by a Tukey’s Post-Hoc test to determine differences among parameters tested.

Short Answer Questions: Short answer questions were analyzed qualitatively by entering student responses into a free-use word cloud generator, TagCrowd, (Steinbock, n.d.) to document high frequency words. Parameters were set so words that did not appear more than one time were excluded.

Results:

Student Learning

Student responses to the multiple-choice question “What percentage of water on Earth is freshwater?” indicated students learned material with exposure to all communication modes. Specifically, a greater proportion of students chose the correct answer more often in the post survey compared to the pre survey (website: $\chi = 61.698$, df = 3, $p < 0.001$; pamphlet: $\chi = 269.799$, df = 3, $p < 0.001$; video: $\chi = 109.004$, df = 3, $p < 0.001$). Across communication modes, ~11% of students answered correctly in the pre-survey whereas ~70% answered correctly in the post survey (Figure 1). However, the proportion of correct responses in the post survey varied depending on the mode viewed (χ = 17.831, df = 6, p < 0.01). Exposure to the website yielded the highest increase in correct student responses (pre = 12% to post = 75%) while the video exposure yielded the lowest increase (pre = 9% to post = 62%). In contrast, the proportion of correct student responses in the 2nd post survey assessing longer-term retention did not differ based on the communication mode viewed (p = 0.320). Exposure to the pamphlet and video yielded the highest amount of correct responses in the 2nd post survey (~32%) whereas the website yielded ~10% fewer correct responses (22%). The video resulted in the lowest change in correct responses between the initial post survey and the 2nd post survey (post = 62%, 2nd post = 32%) and the website resulted in the highest change in correct responses (post = 75%, 2nd post = 22%).

Student Attitudes

Student responses to the question "How concerned are you about water quality?" suggested a change in concern between the pre and post survey with exposure to all communication modes (Figure 2). Mean student response increased by ~1.2 points between the pre and post survey (website: p < 0.008, t = 2.714, df = 129.92; pamphlet p < 0.001, t = 3.6911, df = 229.33; video: p < 0.001, t = 3.5369, df = 206.78). In contrast, responses regarding how often students thought about water use did not change in all modes. Only students who viewed the pamphlet indicated a higher frequency of water use consideration (~0.8 points) in the post survey relative to the pre-survey (p = 0.015, t = 2.4533, df = 232.66). The website and video post survey responses also had a small increase (< 0.6), but neither were significant (website: p = 0.119; video p =0.138)

Changes in student responses to the water-related attitude questions were different among communication modes (ANOVA Figure 3A: F = 3.492, df = 2, p = 0.032; Figure 3B: F = 5.666, df = 2, $p = 0.003$). When asked "How concerned are you about water quality?" student response change was ~0.2 points greater when students viewed the video relative to the students who viewed the pamphlet. In contrast, when asked "How often do you consider water use in daily activities?" student response change was ~0.2 points lower when viewing the video relative to when they reviewed the informational pamphlet. Response change when students viewed the website did not differ from changes associated with exposure to the pamphlet or video.

Student responses for each of the modified NEP statements only differed between the pre and post survey in one statement: "The balance of nature is delicate and easily upset" (Figure 3). Mean response when students viewed both the website and the pamphlet increased by ~0.5 points between the pre and post survey, moving students from the "Unsure" range into the "Agree" range for this question (website: $t = 3.5944$, df = 129.99, $p < 0.001$; pamphlet: $t = 4.3317$, df = 231.58, $p < 0.001$). Mean response for students who viewed the video only increased by ~0.2 and did not differ between the pre and post survey ($p = 0.067$). Responses to the other three NEP statements did not differ between the pre and post survey (Table 2).

Behavior Choices

Student responses to the medicine disposal scenario question indicated students chose the most appropriate environmental behavior more often in the post survey compared to the pre survey only when exposed to the website ($\chi = 9.417$, df = 2, $p = 0.009$) and pamphlet ($\chi = 25.509$, df = 5, $p < 0.001$) but not the video ($p = 0.053$; Figure 5). The pamphlet yielded the highest change in appropriate responses, and the highest appropriate response in the post survey (pre = 30% to post = 60%). Exposure to the website resulted in a lower shift to appropriate response (pre = 13% to post = 40%). Students who watched the video had the highest proportion of appropriate responses in the pre survey and exhibited the lowest change in the post survey (pre = 42% to post = 54%).

In contrast to the medicine disposal scenario, no communication mode affected student response between the pre and post survey in the scenario highlighting positive environmental behaviors associated with choices in transportation (website: $p = 0.118$; pamphlet: $p = 0.065$; video: $p = 0.279$). However, for all three modes of communication, the proportion of student responses that indicated positive environmental behavior in the grocery scenario was higher in the post survey relative to the pre survey (Table 3). The proportion of students that chose to buy recycling bags despite the loss of a bakery treat increased by 30% for those that viewed the website, 20% for those that read the pamphlet, and 19% for those that watched the video.

Short-answer student responses varied in terms of both word choice and frequency of words used between the pre and post survey for each question. Notably, responses to the question: “What are some ways you can help reduce freshwater pollution?” indicated potential shifts in student behavior based on the communication mode viewed. In the post survey for this question, an increase in the use of the word “medicine” was seen for all communication modes (Figure 8). In contrast, key words said audibly in the video, such as “natural and “ingredients”, were used with higher frequency in the post survey only by students who viewed the video although these words were provided as written text in the pamphlet and website. Further, students exposed to the pamphlet or website only minimally altered their word choice for short-answer questions in the post survey relative to students who were exposed to the video that included a broader variety of terms.

Student Perception

Student perception of learning (p = 0.138) and the effectiveness of the activity (p = 0.066) were not different based on the communication mode viewed (Figure 7). For example, when asked, “How effective do you think the activity was at helping you learn information?” and “How much do you feel you learned from this activity?” student responses were not different among the communication modes. Students indicated all communications modes were effective in helping them learn the information. Further, students indicated that they did learn information with all communication modes.

Discussion:

Communication Mode Effects: Learning

Contrary to our hypothesis, the video was not the most effective communication mode to transmit information to students. Rather, the website was the most effective as measured by knowledge retained. This may have been due to differences in how the information was delivered. For example, in the video, students were exposed to both audio and textual information simultaneously at the key scene where information was relayed for the knowledge question tested. Past studies have shown that exposure to text on screen, as well as audio may be distracting and decrease overall learning (Mayer et al. 2001; Lowe et al. 2003; Oberfoell and Correia, 2016). The distraction of multiple elements at once may have decreased student retention of this information. This is further supported by the fact that when students were only exposed to audio and visual images without text, they only reported specific key words when exposed to the video (Figure 8), highlighting the video’s effectiveness in transmitting information outside the knowledge question tested. Alternatively, retention of knowledge may have been higher with website exposure as students could view the information at their own pace as opposed to the single viewing of the video which displayed assessed knowledge information for ~26 seconds. Placement of the knowledge content in the video may have also influenced student retention. In business videos, average engagement declines after 2 minutes (Fishman, 2016). In this study, the knowledge content assessed was relayed after 2 minutes and 28 seconds of viewing time. However, previous assessments of science communication videos have found no correlation between popularity of the video and total video length (Welbourne and Grant, 2016), suggesting that placement of key content in a video to assess learning warrants further study.

Communication Mode Effects: Attitudes and Behaviors

Also inconsistent with our hypothesis, the mode that yielded the greatest shift in attitude was not the video, but the pamphlet. Similarly, the mode that caused the greatest retention (website) did not cause the greatest change in attitude or potential behavior. One possible mechanism for why the video was not the most successful mode for transmitting information or changing attitudes and behaviors may be bias in student reporting. Specifically, students may be engaged in the "social desirability bias" in which they report what they think is correct as opposed to what they would really do (Nederhof, 1985). With environmental issues, subjects may feel pressured to be socially responsible and choose a positive behavior (Michelson, 1990), even though they may only be reporting their intent as opposed to what they would actually do (Olson, 1981). Several studies on the issue of recycling have shown that behavioral intent or self-reported behavior does not necessarily translate into actual materials recycled (Lee, 1993; Corral-Verdugo, 1997). Multiple metrics are likely needed to understand if behavior is affected (Chao and Lam, 2011). In this study, behavioral intent was only measured by asking how students would act in future situations. Differences in responses among modes could be due to students self-reporting positive changes (behavioral intention) that may not translate to changes in actual behavior. Thus, future work in understanding the role modality plays in environmental attitudes and behavior should address not only self-reported behavioral intention, but also actual behavior after exposure to information in variable modes.

Factors such as the music played during the video may also have affected student attitudes. The music chosen was strategic to try and evoke emotions related to concern and empowerment. Changes in emotion in response to music are well documented (Westermann et al. 1996; Morris and Boone, 1998). Thus, our finding that students viewing the video did not have changed attitudes was surprising. This suggests that perhaps the music chosen did not influence students as desired, or if an emotional response was induced, it did not influence behavior and attitude choices.

Communication Mode Effects: Student Preference

Students did not report a preference for communication mode in learning information, which was surprising given the popularity among students for using online sources as opposed to print media (Table 1). Past studies have reported increased success and enjoyment of coursework when videos were incorporated into class (Hsin and Cigas, 2013). In this study, when asked for suggestions students self-reported a desire to view videos as opposed to reading material in the pamphlet and website. The disparity between the lack of the video's effect on knowledge retention, changes in attitudes, and behaviors compared to the other modes, and the perceived enjoyment of videos by students supports the modality theory. For example, while enjoyment may be higher in more dynamic communication such as videos, presenting information visually, audibly, and with text decreases how much is retained by the viewer (Moreno and Mayer, 2007; Oberfoell and Correia, 2016). Nisbet and Scheufele (2009) highlight how understanding the preferred method of communication for an audience is necessary to accurately transmit the intended message. However, we argue that also understanding information processing within communication modes is essential to make sure the information is not only received well, but also understood easily by the audience.

Conclusions:

Research on science communication is a rich field; however, with the emergence of increasingly ubiquitous technology, there is a need to understand how these modalities impact the effectiveness of science communication. Although more dynamic forms of communication such as videos are popular, this study suggests they may not be ideal for transmitting scientific information or altering attitudes and behavior. Placement of content, as well as understanding when diverse media may clutter content focus, are areas to further explore in modality research. In addition, more information is needed on diverse audiences with variable values and beliefs to understand whether contextual information is more influential than modality. Additional research assessing multiple scientific issues may yield insight into whether effects from modality are issue or audience specific. Ultimately, science communicators need to understand what mode of communication is most favored by their audience, as well as what will practically work well to transmit information and translate into positive behavioral changes.

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Table 1. Percentages of all student responses in the pre survey highlighting current behavior choices in communication. In Question 1 students were asked: “Approximately how often do you listen to informative or educational videos online to learn information?” In Question 2 students were asked: “Approximately how often do you read informational websites to learn information about a topic?”

Response	Question 1: Video	Question 2: Website
Daily	18.9	21.8
Weekly	35.9	36.1
Monthly	14.4	16.1
Annually	23.0	18.2
Never	7.8	7.9

Table 2. Response mean and range for the four modified NEP statements. The response mean is an average of the pre and post survey in each communication mode. The range depicts the highest and lowest value seen across the pre and post survey of all modes. Asterisks denote significant difference between pre and post survey responses. W = website, P = Pamphlet, V = Video.

Question		Response Mean		Range	Mean Corresponding Answer
	Mode	Pre	Post		
(1) The balance of nature is delicate and easily upset	W*	3.54	4.06	3.54-4.06	"Unsure to Agree"
	P*	3.58	4.01		
	V	3.78	3.97		
(2) Humans have the right to modify the natural environment to suit their needs	W	2.83	2.81	2.73-3.00	"Disagree to Unsure"
	P	2.96	2.73		
	V	3.00	2.75		
(3) All things in the natural environment are interconnected and dependent on each other	W	3.80	3.88	3.80-4.01	"Unsure to Agree"
	P	3.92	4.00		
	V	4.00	4.02		
(4) Ultimately, there's nothing individuals can do to manage or change the natural environment	W	2.29	2.33	2.29-2.53	"Disagree"
	P	2.52	2.42		
	V	2.43	2.31		

* $p < 0.001$

Table 3. Percentage of each student response for Option 1: the inappropriate environmental behavior and Option 2: the appropriate environmental behavior in the pre and post survey for the grocery store scenario for each mode.

Mode	Choice	Pre Survey	Post Survey	Statistics
Website	1	65.6%	34.4%	χ = 10.949, df =1, p < 0.001
	2	35.3%	64.7%	
Pamphlet	1	65.4%	34.6%	χ = 9.660, df =1, p = 0.002
	2	43.1%	56.9%	
Video	1	63.9%	36.1%	χ = 7.887, df = 1, p = 0.005
	2	42.4%	57.6%	

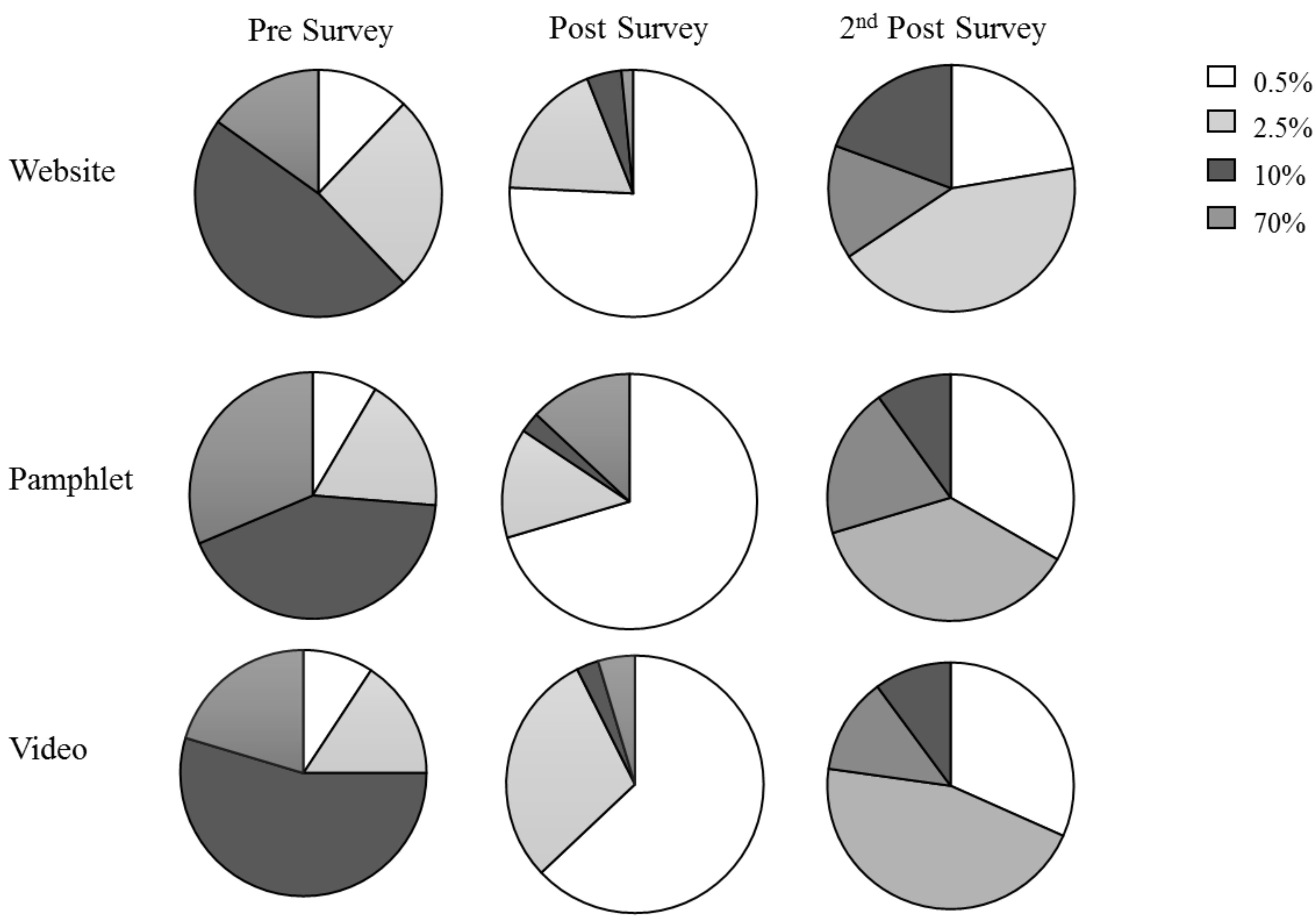
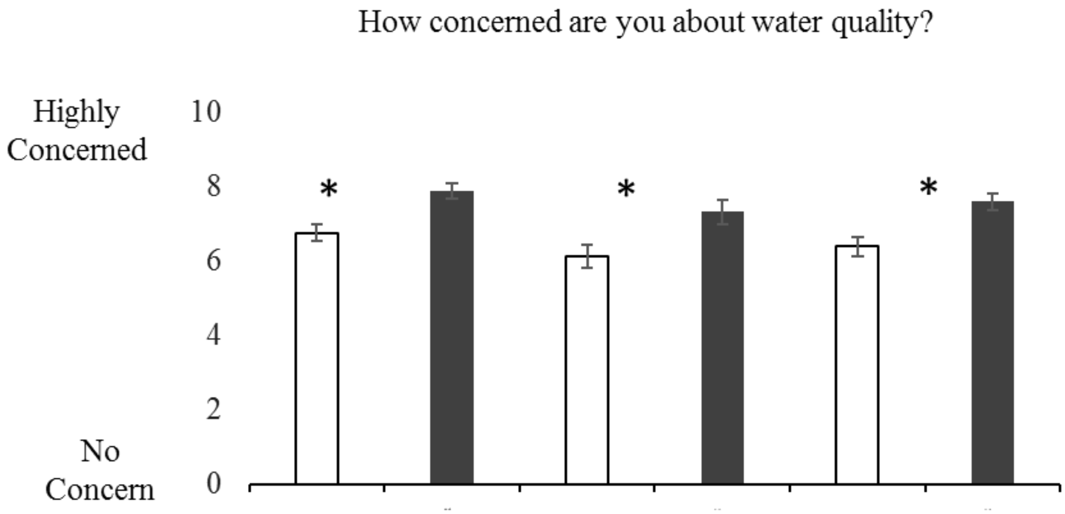


Figure 1. Pre, post, and 2nd post survey responses for the multiple-choice question: "What percentage of water on Earth is freshwater?" Shading denotes the different possible answers. The correct response was 0.5% (white).



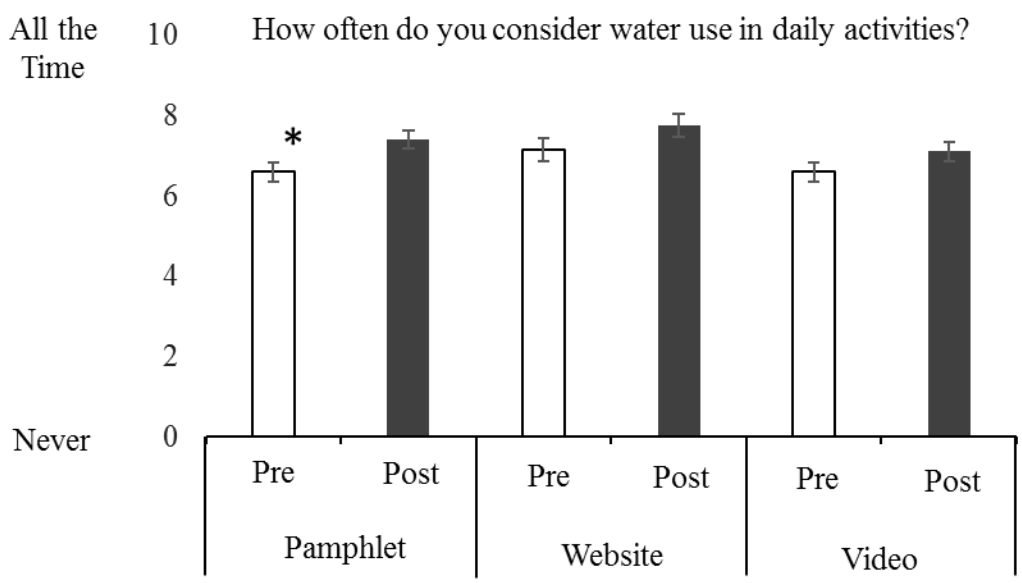


Figure 2. Average student response for two attitude questions: A) "How concerned are you about water quality" and B) "How often do you consider water use in daily activities?" Asterisks denote a significant difference (t-test; $p < 0.05$) in response between the pre and post survey for each communication mode.

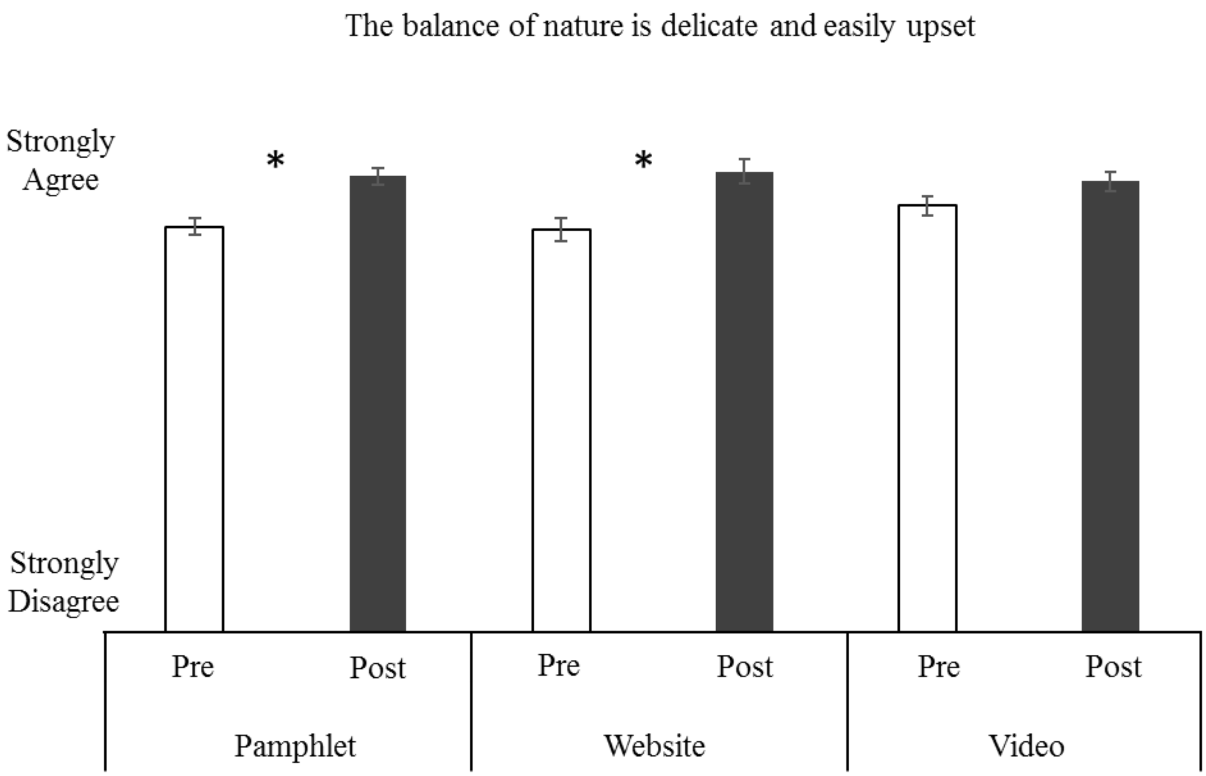


Figure 3. Mean student response scaled from 1-5 where 1 = Strongly Disagree = 1, Disagree = 2, Unsure = 3, Agree = 4, Strongly Agree =5. Asterisks denote a significant difference (t-test; $p < 0.05$) in response between the pre and post survey for each communication mode.

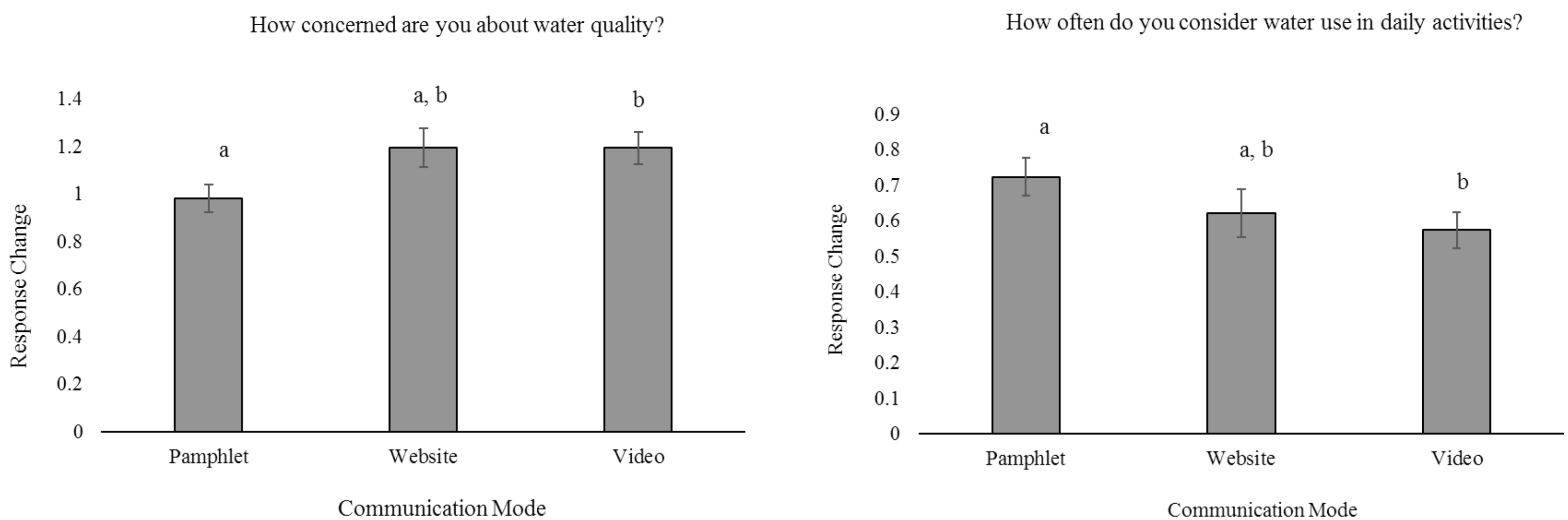


Figure 4. Mean student response change between the pre and post survey for each communication mode for the questions: "How concerned are you about water quality" and "How often do you consider water use in daily activities?" Bars with the same letter denote no significant difference.

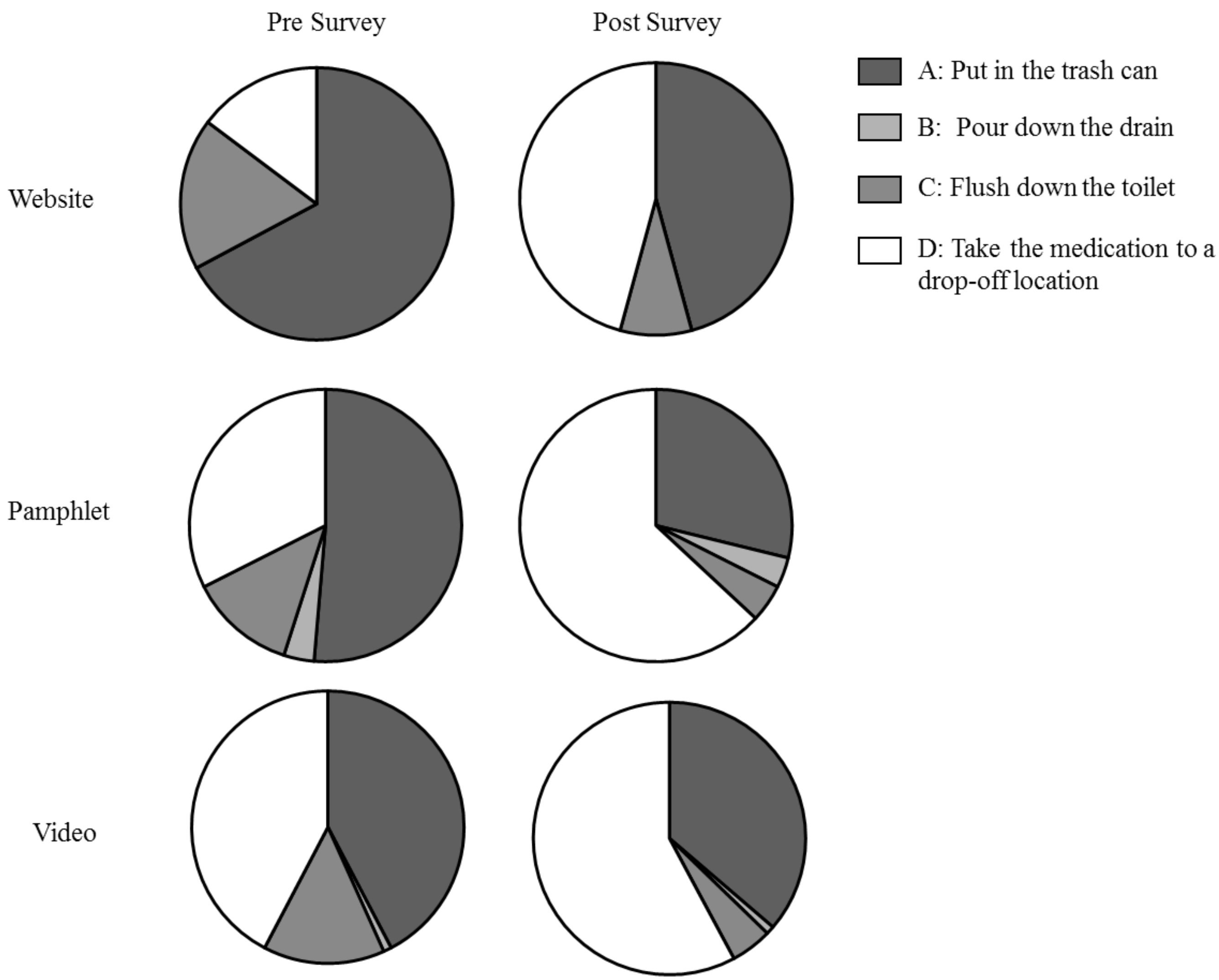


Figure 5. Pre and post survey responses for the scenario question focusing on medicine disposal. Shading denotes the different possible answers. The most appropriate behavior as discussed in the communication modes was D: Take the medicine to a drop off location (white).

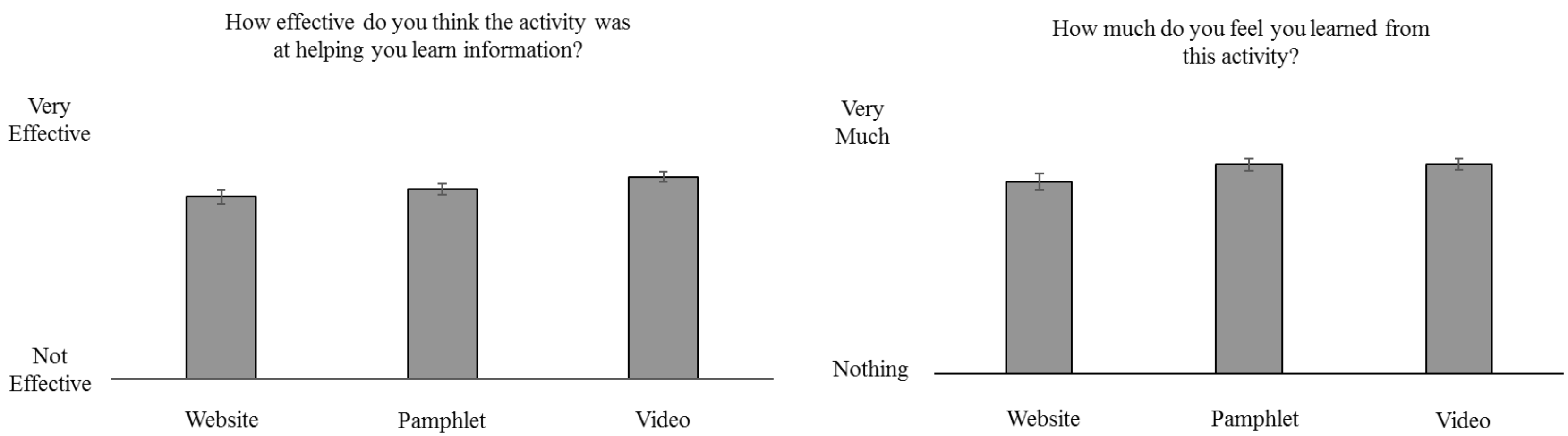


Figure 7. Mean student response in the post survey regarding student opinion on the effectiveness of each communication mode.

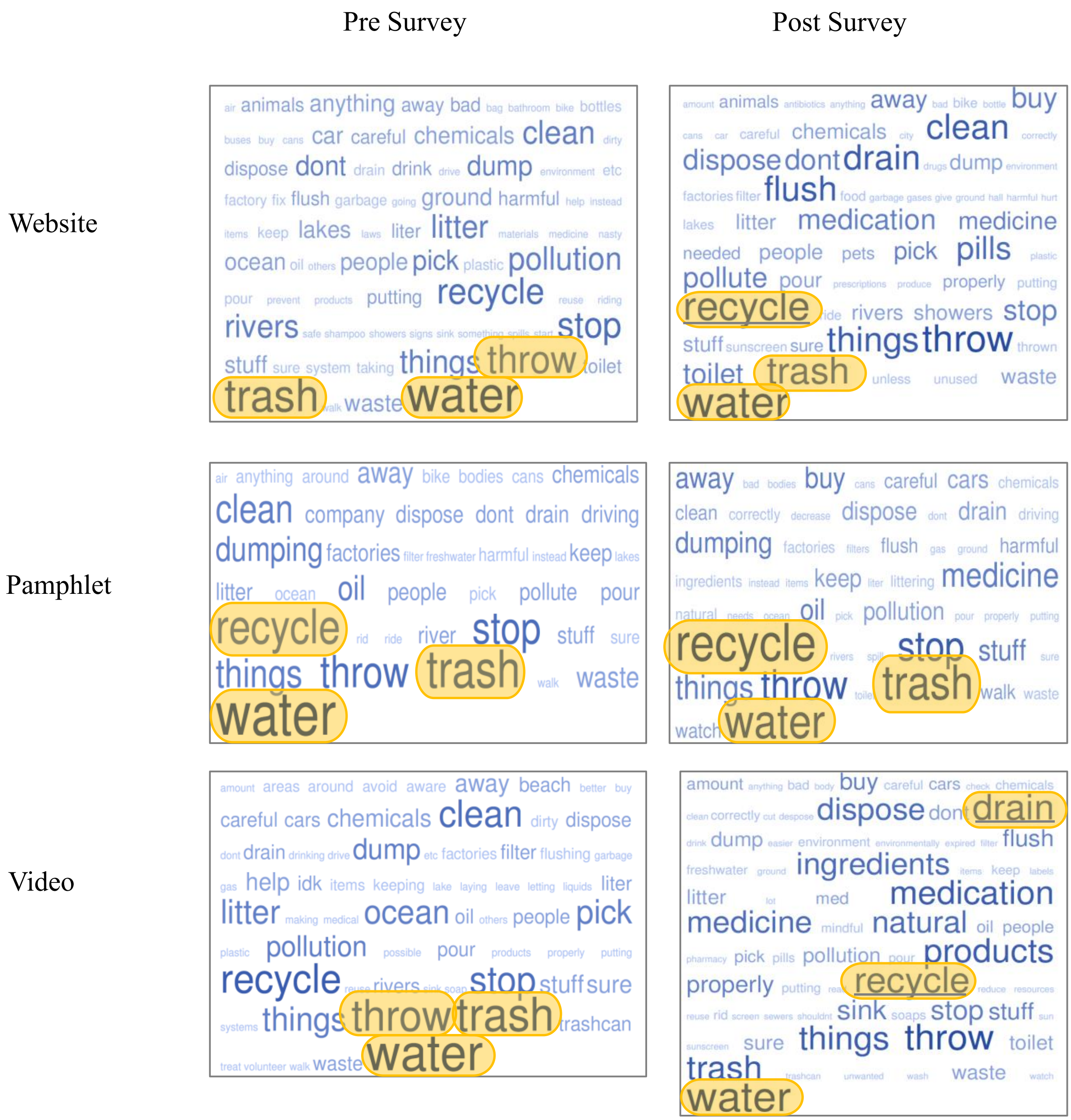


Figure 8. Word cloud visuals of all words appearing at a frequency of 2 or higher in the pre and post survey for the question: “Freshwater pollution is a problem in many parts of the world. What are some ways you can help reduce freshwater pollution?” Highlighted words were the top three most frequent words used by students in both the pre and post survey. Underlined words indicate words that were not in the top three words in the pre survey, but were in the post survey.

Chapter #2: Effects of trace organic contaminants on algal community structure and function

Introduction:

Freshwater is essential to organismal survival and human needs. However, water quality is altered by human activities including agricultural and urban development. For example, excess nutrients from agricultural runoff create algal blooms that lead to hypoxic or anoxic conditions in aquatic ecosystems (Paerl, 1988; Hallegraeff, 1993; Nixon, 1995). Though the abundance and effects of some contaminants are relatively well studied, novel contaminants are continually being documented in freshwater resources that threaten aquatic organisms and human health.

One emerging group of contaminants are trace organic compounds (TOCs) which include antimicrobials, sunscreens, antibiotics, fragrances, and prescription drugs (Kolpin et al. 2002; Murray et al. 2010; Boxall et al. 2012; Rosi-Marshall and Royer, 2012). These compounds enter aquatic ecosystems primarily through wastewater as well as through industrial and agricultural waste streams (Boxall et al. 2012). Concentrations of TOCs vary with surrounding land use and time of year (Kolpin et al. 2002; Bunch and Bernot, 2011; Veach and Bernot, 2011). Nationwide, specific TOCs of concern include N,N-Diethyl-3-methylbenzamide (DEET), carbamazepine, acetaminophen, triclosan, and sulfamethoxazole due to their high abundance and persistence in the environment (Boxall et al. 2012; Murray et al. 2010; Veach and Bernot 2011).

Although the abundance of TOCs in freshwaters has been well established (Kolpin et al. 2002, Bernot et al. 2016), compound use and degradation by aquatic organisms is not yet understood (Boxall et al. 2012), particularly across trophic levels. Laboratory studies have yielded insights into potential effects on vertebrates. For example, exposure to metformin (diabetes prescription) at concentrations found in wastewater effluent can cause feminization of male fathead minnows, as well as reduced size and overall fecundity (Niemuth and Klaper, 2015). The American Toad, *Bufo americanus*, has reduced survivorship when exposed to triclosan and acetaminophen; however, caffeine does not affect mortality of *B. americanus* (Smith and Burgett, 2005). Additionally, TOCs can affect species diversity of invertebrates. For example, carbamazepine may influence macroinvertebrate richness in Indiana streams (Jarvis et al. 2014).

In contrast to other trophic levels, effects on algae are less understood. As primary producers, algae have the potential to drive both community structure and function in aquatic ecosystems through trophic interactions (Minshall, 1978). For example, pesticide effects on algal biofilm structure have influences on higher trophic levels by altering consumer fitness (Feckler et al. 2015). Understanding the effects of TOCs on algae is vital to elucidating how these compounds may affect whole ecosystems.

Research has shown TOCs have variable effects on algal dynamics depending on species and ecosystem. With *in situ* exposure, diphenhydramine and ciprofloxacin can suppress algal biomass and gross primary production; however, caffeine can stimulate gross primary production (Shaw et al. 2015), as well as suppress algal biomass (Rosi-Marshall et al. 2013) depending on conditions. Laboratory exposure to TOCs can elicit changes in community structure (Wilson et al. 2003). Additionally, *in situ* exposure to both TOCs individually or as a mixture can result in decreased biomass, metabolism and altered species abundance (Rosi-Marshall et al. 2013). Further, synergistic effects of multiple compounds can yield toxicity to primary producers (Backhaus et al. 2011). As many TOCs have not yet been studied, assessments of the effects of TOCs on algal function and community structure are warranted.

In this study, we aimed to understand the effects of five commonly found TOCs on algae community structure, and overall function through both *in situ* and *in vitro* manipulations. We hypothesized that exposure to TOCs would alter algal dynamics. Specifically, we hypothesized that exposure to TOCs would decrease algal biomass as well as metabolic activity and net nutrient assimilation. Additionally, it was hypothesized that exposure to TOCs would alter community structure by decreasing species diversity.

Methods:

Study Design

To quantify effects of TOCs on algal dynamics, both *in situ* and *in vitro* experiments were performed incorporating the same concentration of TOCs (Table 1). Both experiments exposed algae to target concentrations over an incubation period with subsequent measurement of algal dynamics. The *in situ* experiment assessed the differences in algal growth, community structure, and final metabolism with exposure to TOCs in the context of a stream environment. The *in vitro* experiment also quantified the effects of TOC exposure on growth and metabolism. However, this experiment utilized established filamentous algal communities in laboratory conditions. In addition to algal growth and metabolism, the *in vitro* experiment also quantified nutrient assimilation.

Study Site

Algae and water sampling, as well as field incubations, were conducted at Morrow's Meadow, at a tributary to the White River in east-central Indiana. This study site was chosen because of its accessibility and overall depth and streambed type necessary for attaching substrates for stream incubation.

Target Compounds

The compounds selected for this study were 1) acetaminophen, an over the counter drug used for treating pain, 2) sulfamethoxazole, a commonly prescribed antibiotic, 3) carbamazepine, an epileptic medication, 4) triclosan, a disinfectant, and 5) N,N-Diethyl-meta-toluamide (DEET), an ingredient found in insect repellent. These compounds were selected for their abundance in freshwaters measured in both national studies (Kolpin et al. 2002; Bernot et al. 2016), and regional studies (Veach and Bernot, 2011). Additionally, selected compounds represent a range of chemical classes that comprise trace organic contaminants. Treatment concentrations were selected to represent concentrations comparable to effluent input of compounds in freshwater ecosystems (Hirsch et al. 1999; Petrie et al. 2015; Baldwin et al. 2016).

Stock Solutions

Stock solutions for substrate treatments were made by dissolving compounds (mg) in deionized water, except for carbamazepine, which required an addition of methanol (3.0%) for compound dissolution. Specifically, an ampule of 100 mg/L DEET Neat solution (Ultra Scientific, PST-298) was added to 1 L of deionized water; 1 mg of acetaminophen (Sigma-Aldrich, 103-90-2), triclosan (AccuStandard, Inc., 3380-34-5), and sulfamethoxazole (Fluka, 723-46-6) were dissolved in one liter of deionized water. Ten milligrams of carbamazepine (Sigma-Aldrich, 298-46-4) were added to a 3.0% methanol solution (30 mL) supplemented with deionized water (970 mL). Stock solutions were made fresh for both the *in situ* and *in vitro* experiments. For *in vitro* stock solutions, all compounds were dissolved in 5.0% methanol to ensure complete dissolution. Experimental treatments were applied by transferring appropriate volumes of stock solutions into mesocosm solutions.

In situ Experiment - Pharmaceutical Diffusing Substrates (PhDS)

To assess differences in algal community structure and function with *in situ* exposure to TOCs, PhDS were created similarly to Nutrient Diffusing Substrates (NDS) by enriching agar solutions with each target compound (*sensu* Tank et al. 2006). Agar solutions were made by adding approximately 20 g of Agar (Acros Organics, 9002-18-0) to one liter of boiling, deionized water to create a 2% by weight Agar solution. Agar was added slowly over two hours with constant stirring to create a homogenous solution without clumps. Specific volumes of stock concentrations were then added to bring the final solution to the desired treatment concentrations (Table 1). The control solution was only agar with no addition of a compound. Agar solutions were poured into plastic cups (~30 mL) and allowed to cool before being fitted with a fritted glass disc (diameter: 2.86 cm). Each treatment, including the control, had 10 replicate cups. All replicates were cooled and wrapped individually with plastic-wrap until field deployment (< 1 week).

PhDS were attached to metal L-bars (61 cm by 5.1 cm by 5.1 cm) and deployed in the White River on January 28th, 2016 by securing to the benthos. Cups were fully immersed in water with ~ 0.2 m water depth. PhDS were exposed to stream conditions for 19 days. During incubation, PhDS were rinsed on two separate days to eliminate sediment build-up. Rinsing consisted of removal of PhDS cups momentarily followed by gentle re-submerging to dislodge sediment and subsequent re-securing on the benthos. Stream characteristics were measured and water samples were collected at the beginning and end of the incubation period to quantify field conditions. Water samples for TOCs were collected following methods described in Bernot et al. (2013) and were frozen until transport (one week) to the Indiana State Department of Health (ISDH) Chemistry Laboratory. At the ISDH, water samples were analyzed for 24 trace organic compounds including: acetaminophen, albuterol, caffeine, carbamazepine, chloramphenicol, codeine, cotinine, DEET, diphenhydramine, gemfibrozil, ibuprofen, lincomycin, naproxen, paraxanthine (1,7-Dimethylxanthine), sucralose, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole sulfathiazole, trimethoprim, triclocarban, triclosan and tylosin. All TOC concentrations were measured through solid-phase extraction liquid chromatography tandem mass spectrophotometry (SPE/LC/MS/MS: Applied Biosystems triple quad API 4000 equipped with an Agilent 1200 HPLC). Extraction and analysis followed established protocols (USGS Method 5-B5; Cahill et al. 2004; Furlong et al. 2008). Additionally, 125 mL of water was collected in duplicate acid-washed Nalgene© bottles for analyses of major anion and cation concentrations. Ions analyzed were nitrate (NO_3-N), phosphate (PO_4-P), chloride (Cl^-), sulfate (SO_4^{2-}), ammonium (NH_4-N), potassium (K^+), magnesium (Mg^{2+}), fluoride (F^-), sodium (Na^+) and calcium (Ca^{2+}) using ion chromatography (DIONEX, ICS-3000).

Glass discs were collected from the field on February 16th after 19 days of incubation by individually removing and placing each disc into separate, labeled Ziploc bags. Fritted discs were immediately placed on ice in a cooler for transport back to the laboratory. At the laboratory, the fritted discs were sorted by treatments and the first randomly selected six discs were analyzed for metabolic rates and chlorophyll *a* concentration while the remaining four discs were analyzed for ash free dry mass (AFDM) and community composition.

Metabolism and Chlorophyll a

Metabolism and chlorophyll *a* were analyzed on the PhDS to determine the effects of the TOCs *in situ* on the growth (chlorophyll *a*) and function (metabolism) of algal communities. The six fritted discs from each treatment (N = 36) were placed into separate Biological Oxygen Demand (BOD) bottles (300 mL) for measurement of metabolic rates (McCormick et al. 1998). Bottles were incubated in an AlgaeTron AG130-ECO for metabolism assays. Dissolved oxygen was measured immediately with a Hach HQ 40 LDO probe following placement of discs into each bottle, one hour following constant infrared and blue/green light, and one hour following complete darkness. Gross primary production was determined as the difference between oxygen concentrations of BOD bottles after exposure to light conditions, and dark conditions. Respiration was the difference in initial oxygen concentration and after exposure to dark conditions. Net productivity was the difference between initial oxygen concentrations and after light exposure. Following light and dark incubations and oxygen measurements, water in the BOD bottles, including the fritted disc, was then vacuum filtered onto a 47 mm Whatman Filter (0.7 micron pore size). Filters and fritted discs were then frozen for chlorophyll *a* analysis.

To quantify chlorophyll *a* concentration, filter discs were extracted in 90% buffered acetone for 24 h followed by measurement of the extract on a UV1700 PharmaSpec. Chlorophyll *a* concentrations were measured at wavelengths of 750 nm and 664 nm. Samples were then acidified by adding 100 µL of 0.1 N HCl and again measured on the spectrophotometer at 750 nm and 665 nm (Steinman et al. 2006). Final concentrations were calculated using the following equation (Steinman et al. 2006):

$$Chlorophyll\ a\ \left(\frac{\mu g}{cm^2}\right) = \left(\frac{26.7\ (E664b - E665a) * (Vext)}{area\ of\ substrate}\right) * L$$

Where E664b = Absorbance of sample at 664nm − Absorbance of sample at 750nm− before acidification);

E665a = Absorbance of sample at 665nm − Absorbance of sample at 750nm after acidification);

Vext = Volume of 90% acetone used in the extraction (mL);

L = length of path light through cuvette (cm);

26.7 = absorbance correction (derived from absorbance coefficient for chlorophyll *a* at 664 nm [11.0] × correction for acidification [2.43]);

1.7 = maximum ratio of E664b: E665a in the absence of pheopigments

Final measurements of chlorophyll *a* (mg/cm^2) were extrapolated to represent measurements of the entire fritted disc.

AFDM and Genera Composition

AFDM was analyzed to provide another measure of the effects of TOCs on algal growth. The four fritted discs from each treatment (N = 24 discs total) were scrubbed into a plastic container using a fresh toothbrush and rinsed with deionized water to create a 100 mL slurry (Wyatt et al. 2008). An aliquot (50 mL) of homogenized slurry was then filtered onto a 47 mm Whatman filter (0.7 micron pore size) for measurement of dry mass via drying at 60°C and subsequent combustion for ash mass at 500°C and calculation of AFDM (Steinman et al. 2006).

The remaining 50 mL slurry aliquot was preserved with 15 drops of 10% formalin and subsequently analyzed for taxa composition (N = 18) to understand how TOC exposure may influence community structure. Specifically, all algae were analyzed by counting 25 fields of view within a Palmer Counter Cell (Biggs and Kilroy, 2000) and were identified to the genus level at 400x magnification following Wehr and Sheath (2003), Briggs and Kilroy (2000), and cross-referencing with Spaulding et al. (2010). The 25 fields of view were randomly counted by generating field of view coordinates. Filamentous cyanobacteria were counted as individual cells, and not as filaments. Of the eighteen replicates counted, 15% of replicates were counted again using different sub-samples for quality control. Re-counted samples had an average difference of less than 1.5 % among total abundance of genera in each sample. Total cell density (mg/cm^2) on fritted discs was quantified as all cells counted following calculations in Briggs and Kilroy (2000), and was extrapolated to include the cell density of the entire fritted disc.

For diversity assessments, percentage of genera in each sample was also calculated using all cells. To compare algal groups and specific genera among treatments, only genera with greater than 5% abundance were analyzed.

In vitro Experiment – Filamentous Algae Mesocosms

To understand differences in algal growth and metabolism with TOC exposure, parent cultures of filamentous algae, predominantly *Cladophora* and *Oedogonium,* were collected from the field and grown in the laboratory in river water for several weeks for mesocosm experiments. Following growth and development of the parent cultures, ~2 g (wet weight) homogenized parent culture were added to 250 mL deionized water in Erlenmeyer flasks (250 mL) for experiments (N = 30). Each flask was also supplied with 32 µL of Algae Food (Aquatic Eco-Systems, Inc.) nutrient mix A (Lot: 50254) and B (Lot: 40274). Six replicate subsamples of 250 mL of deionized water supplemented with nutrients were collected to quantify initial nutrient concentrations. Initial nutrient samples were frozen for subsequent analyses. Experimental flasks were then enriched with target compounds at variable concentrations as for *in situ* experiments (Table 1) with five replicates per treatment. Mesocosms were placed into an AlgaeTron AG130-ECO for a 9 day incubation period under 14:10 light:dark photoperiods and ambient air temperature of 25°C. Replicates were distributed evenly among top and bottom shelves so that no treatment would receive more or less light. Dissolved oxygen measurements were taken with a Hach HQ 40 LDO probe on the first two days of the experiment to document growth conditions.

Following the incubation period, 40 mL of water from each mesocosm was filtered onto a 25 mm Whatman Filter (0.7 micron pore size) mounted to a syringe and frozen for subsequent nutrient analysis, to quantify differences in nutrient assimilation with TOC exposure, Algae from each flask were isolated by passing through a 63-micron sieve and then weighed for wet mass. Weighed algae were then transferred to a BOD bottle (300 mL) containing deionized water for measurement of metabolic rates as previously described; however, algal communities in BOD bottles for the *in vitro* experiment were exposed to the light and dark conditions for eight hours each. A production to respiration ratio (P/R) in the *in vitro* experiment was determined by dividing production by respiration.

Nutrient Analyses

To quantify effects of TOCs on nutrient assimilation, changes in nutrient composition of the water over the experiment duration were determined for both phosphorus and ammonium. The filtered water from each mesocosm (N = 30), and each initial nutrient sample (N = 6) was analyzed for soluble reactive phosphorus (SRP) (Modified from APHA 4500-P E, 1995) and ammonium concentrations (Modified from APHA, 1995; Aminot et al. 1997). For SRP analyses, a stock solution of 100 mg/L KH_2PO_4 was made to create six standards. Each standard was read in duplicate with three standards replicated a third time for quality control. Four reagents (sulfuric acid, antimony potassium tartrate, ammonium molybdate, and ascorbic acid) were mixed in a 100:10:30:60 mL ratio, respectively. A sulfuric acid solution was made by combining 500 mL deionized water with 70 mL of H_2SO_4. Approximately 0.34 antimony potassium tartrate was added to 250 mL of deionized water. Twenty grams of ammonium paramolybdate was added to 500 mL of deionized water, and finally approximately 1.8 g ascorbic acid was added to 100 mL of deionized water. Mixed reagent (3.33 mL) was added to each 15 mL standard and sample. All samples and standards were analyzed after five minutes, but not more than thirty minutes, on a UV1700 PharmaSpec at 880 nm. Final SRP concentrations were calculated by using the equation generated by the standard curve.

Ammonium analyses were also conducted on aliquots of the filtered water from each mesocosm and initial nutrient replicates. A stock solution of 1 mg/L of NH_4CL was used to create six standards ranging from 2 – 200 µL (Modified from APHA, 1995; Aminot et al. 1997). Quality control checks were the same as in the SRP analysis. Reagent A consisted of approximately 0.05 g sodium nitroferricyanide dehydrate mixed with 3.5 g phenol in 100 mL of deionized water. Reagent B was created by combining approximately 20 g of sodium citrate with 2.0 g sodium hydroxide into 200 mL of deionized water. Immediately before use, 15 mL of Reagent B was mixed with 5 mL of fresh bleach to create a 20 mL solution. Each 10 mL sample solution received 385 µL of both Reagent A and B. All samples were then incubated for 18 hours in the dark. Absorbance for each solution was read on the UV1700 PharmaSpec at 630 nm. Final ammonium concentrations were calculated from the standard curve generated.

Statistical Analysis and Calculations

Cell density was calculated following Biggs and Kilroy (2000) where the area of the sample was the scrubbed fritted disc described above. All cells counted were included in analysis of cell density. Variation in cell density among treatments was compared using an ANOVA ($\alpha = 0.05$).

Diversity indices were calculated by determining the percentage of genera abundance out of total cells counted in the sample. All genera were counted in each sample, regardless of genera abundance, to quantify species richness (S), Shannon’s Diversity Index (H), Simpson’s Diversity Index, and evenness (J) following Krebs (1999). Treatment differences for all indices were compared using an ANOVA ($\alpha = 0.05$). Additionally, differences among treatments in metabolism metrics were analyzed using ANOVA ($\alpha = 0.05$), as well as differences among treatments in AFDM, and chlorophyll *a* concentrations.

Phosphorus uptake and ammonium output were calculated by comparing the final SRP and ammonium concentrations of each sample to average concentration of the six initial nutrient samples. Growth over the course of the *in vitro* incubation, as well as the net change in phosphorus and ammonium concentrations were compared using ANOVA ($\alpha = 0.05$).

In all analyses, when significant differences were identified with an ANOVA, they were followed by a Tukey's Post-Hoc test to determine specific differences among treatment groups. All statistical analyses were conducted using R Statistical Software (R Core Team, 2016).

Results:

In Situ Conditions

Average stream temperature during the *in situ* experiment was 2.5°C (1.3 – 3.8). Dissolved oxygen was ~15 mg/L, and pH was 8.3 on the first and last day of incubation. Average depth of the reach was 0.31 m (Table 2). Rainfall during the study period was ~1.3 cm. Five days after incubation start, a large rain event occurred that submerged the PhDS in ~0.5 m of water for two days, obstructing sunlight and increasing river flow. All target enrichment compounds, except triclosan, were detected in field samples at measurable levels ranging from 11 to 342 ng/L (Table 1).

Algal Growth - PhDS

Across treatments, algal biomass at experiment end ranged from 0.17 to 0.53 mg/cm^2 AFDM and 0.00 to 0.01 mg/cm^2 chlorophyll *a* concentrations. Cell density ranged from 13,356 to 39,197 cells/cm^2 across all samples. Algal growth did not vary among treatments when measured as AFDM ($p = 0.585$) or as chlorophyll *a* concentration ($p = 0.338$). However, cell density did vary among treatments (Figure 1; $p = 0.011$). Specifically, cell density on fritted discs exposed to acetaminophen (mean = 34,126± 5,639 cells/cm^2) and carbamazepine (mean = 29,828± 6913 cells/cm^2) was ~18,000 and ~14,000 cells/cm^2 higher, respectively, relative to controls (mean = 15,528± 1608 cells/cm^2). While cell density on fritted discs exposed to sulfamethoxazole, triclosan, and DEET had a mean cell density ~5,000, ~9,000, and ~12,000 cells/cm^2 higher than controls, this difference was not significant.

Algal Community Composition - PhDS

Overall, algal community composition was composed of ~75% diatoms, ~10% green algae and ~5% cyanobacteria across treatments (Figure 2). Percentage of diatoms varied among treatments (ANOVA, $p = 0.014$), with fritted discs treated with triclosan, sulfamethoxazole, and carbamazepine having higher diatoms abundance relative to discs exposed to DEET. Specifically, the lowest abundance of diatoms was 50% in the DEET treatment, and the highest diatom abundance was 81% in the carbamazepine treatment. Carbamazepine and triclosan exposed replicates contained ~7% and ~6% more diatoms, respectively, relative to controls. Green algae did not vary among treatment groups (ANOVA, $p = 0.266$). Fritted discs treated with DEET had ~13% more green algae than fritted discs treated with acetaminophen, which exhibited the lowest average green algae abundance (mean = 5.19%). Additionally, abundance of cyanobacteria did not vary among treatments (ANOVA, $p = 0.533$), with only two treatments (acetaminophen and DEET) having any cyanobacteria cells.

Diatom genera abundance did not vary among treatments for most genera present. However, mean *Nitzschia* abundance was different among treatment groups (ANOVA, $p < 0.001$). *Nitzschia* abundance ranged from 0% to 16% across samples and was ~8% higher in all treatments (mean = 8.4%) relative to controls (0%) with the exception of discs exposed to DEET (0%) (Figure 3). Also, *Neidiomorpha* abundance varied among the treatment groups (ANOVA, $p < 0.001$). Only the controls and sulfamethoxazole treatment contained any *Neidiomorpha* cells.

Species genera richness (S) ranged from 8 - 18 total genera. Shannon's Diversity Index (H) was 2.54 to 3.64, and Simpson's Diversity Index varied by 0.08 among samples with a range of 0.81 to 0.89. The degree of Evenness (J) among samples varied from 0.75 to 0.88. Diversity metrics of S ($p = 0.866$), H ($p = 0.193$), and Simpson's ($p = 0.071$) did not vary among treatments. In contrast, community evenness did vary among treatments (ANOVA, $p = 0.034$), with the algal communities exposed to acetaminophen being less even (mean = 0.79) than algal communities from control fritted discs (mean = 0.87) (Figure 4).

Algal Function - PhDS

Across treatments, net primary production ranged from -0.40 to 0.29 mg/L of oxygen and net respiration ranged from -0.34 to 0.59 mg/L of oxygen. As a result, net production ranged from -0.52 to 0.36 mg/L of oxygen. Gross primary production ($p = 0.270$), respiration ($p = 0.333$), and net productivity ($p = 0.488$) did not vary among the treatments. The highest gross primary production (mean = 0.11 mg/L) was associated with acetaminophen exposure; whereas, the lowest (mean = -0.11 mg/L) was associated with DEET exposure (Figure 5). Similar trends were observed in net productivity, but not in respiration.

Filamentous Algae Growth - Mesocosms

A majority of mesocosms (87%) had measurable algal growth over the incubation period, which ranged from 0.00g to 2.33 g. However, growth did not vary among treatments (ANOVA, $p = 0.395$). Overall, control mesocosm had the highest growth (mean = ~1.0 g) and exposure to carbamazepine had the lowest growth (mean = ~0.2 g) (Figure 6).

Filamentous Algae Function - Mesocosms

No differences were exhibited among the metabolism metrics of mesocosm communities (Figure 7). Gross primary production ranged from 0.33 to 4.89 mg/L of oxygen, but did not differ among treatments (ANOVA, $p = 0.072$) Exposure to DEET yielded the highest gross primary production in mesocosm (mean = 3.01 mg/L), whereas exposure to carbamazepine yielded the lowest gross primary production (mean = 1.35 mg/L). Respiration varied from 0.19 to 4.63 mg/L of oxygen. Respiration was also highest in the mesocosms treated with DEET (mean = 2.09 mg/L), with no overall differences among groups (ANOVA, $p = 0.113$). In all treatments, production to respiration ratios (P/R) were above one on average, indicating that algal communities had higher production than respiration (Figure 7); however, P/R ratios did not vary among treatments (ANOVA, $p = 0.164$).

Filamentous Algae Nutrient Assimilation - Mesocosms

Net phosphorus uptake ranged from 2.05 to 5.80 µg PO_4^{3-} L/h. Net ammonium uptake ranged from -0.02 to 3.29 µg NH_4^+ /L/hr. However, differences in net nutrient uptake for phosphorus ($p = 0.586$) and ammonium ($p = 0.438$) did not vary among treatments. General trends of nutrient assimilation were different between phosphorous and ammonium. For example, mesocosms exposed to DEET had the highest net phosphorus uptake (mean = 5.42 µg PO_4^{3-} L/h) while mesocosms exposed to triclosan had the lowest net uptake (mean = 4.43 µg PO_4^{3-} L/h). Net ammonium uptake was highest in mesocosms exposed to triclosan (mean = 1.60 µg NH_4^+ /L/hr), whereas those exposed to carbamazepine had the lowest net ammonium uptake (mean = 0.37 µg NH_4^+ /L/hr).

Discussion:

Algal Growth

Contrary to the hypothesis that exposure to TOCs would decrease algal biomass, algal biomass did not differ with TOC exposure in either the *in situ* or *in vitro* experiments. However, when measured as cell density, *in situ* exposure to acetaminophen and carbamazepine increased total number of algal cells. Exposure to ambient stream conditions during the *in situ* incubation, such as light limitation associated with sediments, may have suppressed some algal growth and yielded either opportunistic growth of some genera or toxicity to others that was more apparent. Sediment interactions with algal response are evidenced by the proportion of AFDM relative to chlorophyll *a* measured on substrates (Figure 1). Thus, some of the material was likely non-photosynthetic. While counting methods addressed algal filaments as individual cells, even when only counting filaments based on 10 μm units, differences were still seen among treatments (*data not shown*, $p = 0.027$). Additionally, exposure to acetaminophen (Lawrence et al. 2011), and carbamazepine (Andreozzi et al. 2002) did not suppress algal biomass in other laboratory studies. Algal response to trace organic contaminants in natural stream ecosystems is likely a function of multiple physiochemical factors also driving growth (Dodds et al. 2002).

Over the nine-day *in vitro* incubation, ideal growth conditions were documented through increased oxygen output during the first two days of the incubation (*data not shown*). However, TOC concentrations may have declined during the incubation period potentially affecting growth response. Mesocosm TOC treatments were added only at experiment start. Triclosan is sensitive to photolysis with a documented half-life of as little as one hour (Lindström et al. 2002) and as much as five hours (Latch et al. 2005) in natural lake ecosystems. As a result, triclosan concentrations were likely reduced by the end of the first day and results do not represent chronic exposure to compounds at the target concentration. Similarly, approximately 45% of sulfamethoxazole and 5% of carbamazepine concentrations can be removed after 4.5 days of exposure to biodegradation through bacterial cultures (Vasiliadou et al. 2013), resulting in a possible 90%, and 10% decrease in sulfamethoxazole and carbamazepine concentrations respectively by the end of the experiment. Other degradation studies propose degradation rates under multiple exposure techniques (addition of H_2O_2, pH = 3, etc.); however, as only photolysis and exposure to algae was a factor in this study, those rates are not compared here (Vogna et al. 2004; Martinez-Haya et al. 2017). Variable degradation rates could explain why *in vitro* effects on growth did not differ from the control group.

Community Structure

Community structure was affected by TOC exposure, consistent with the hypothesis. While species richness (S) did not differ among treatments, genera groups, such as *Nitzschia,* had varied abundance and evenness that was dependent on treatment. At concentrations as low as 120 ng/L, triclosan has previously been found to alter genera abundance (Wilson et al. 2003), consistent with the results of this study. However, while acute exposure to triclosan causes shifts in community structure, chronic exposure may not yield the same result (Proia et al. 2011). Differential algal response with acute or chronic exposure may be explained by adaptations and resistance to triclosan occurring in chronically exposed algal communities (Drury et al. 2013). The *in situ* study site had dissolved triclosan concentrations either below detection or < 60 ng/L based on measurements made during the incubation and previous studies (Table 1; Veach and Bernot, 2011). However, all other target compounds were detected at concentrations > 11 acetaminophen; 20 carbamazepine; 20 DEET; 232 sulfamethoxazole ng/L. Fritted discs exposed to triclosan had the second highest diatom abundance, only second to carbamazepine which is also considered to be recalcitrant in the environment, highlighting that past exposure to TOCs may play a role in community composition. Future work should incorporate history of TOC exposure to fully understand algal response.

These data show algal communities respond differently depending on specific TOC compound exposure. Algae have been used as ecological indicators for a variety of environmental conditions as well as to provide frameworks for ecological assessment and management (Stevenson, 2006). Differences in community structure, particularly with *Nitzschia*, a genus historically tolerant of pollution conditions (Palmer, 1969; Thomas and Seibert, 1977; Silva-Benavides, 1996), highlights a potential for algae as ecological indicators of TOCs, although more work needs to be done to understand this application.

Metabolic Activity and Nutrient Assimilation

Metabolic activity in both the *in situ* and *in vitro* experiments did not change with TOC exposure, contrary to the original hypothesis. This is in contrast to other studies that suggest TOC exposure can either suppress or stimulate gross primary production. For example, *in situ* exposure to diphenhydramine can suppress gross primary production; however, exposure to caffeine can stimulate production (Shaw et al. 2015). Similarly, *in situ* exposure to TOCs across geographic sites, including sites in Indiana, showed suppression of gross primary production (Rosi-Marshall et al. 2013). Previous *in situ* studies were performed during spring, in contrast to the winter incubation done in this study. TOC concentrations are typically higher in winter (Veach and Bernot, 2011; Vieno et al. 2005) and response may vary with season. In winter, when algae are limited by both light and temperature, gross primary production is inherently lower relative to spring and summer. Thus, small differences in already low gross primary production rates may not be significant in winter. Further work should be done to characterize whether time of year influences *in situ* responses to TOCs.

Exposure to TOCs also did not affect phosphorus or ammonium assimilation rates. While research on algal assimilation rates with TOC exposure is minimal, some *in vitro* research suggests decreased phosphate uptake with exposure to triclosan (Proia et al. 2011). In this experiment, triclosan exposure did yield the lowest average phosphorus uptake, but rates were not different from controls or any other compound. When algal communities are exposed to triclosan with acute pulses, diatom mortality can increase in addition to phosphorus assimilation rates decreasing for up to two weeks (Proia et al. 2011), which further supports degradation of compounds during the experiment. Due to the short half-life of compounds such as triclosan (Lindström et al. 2002; Latch et al. 2005), chronic exposure may not have been achieved, and algal communities could have recovered from initial exposure quickly enough to limit influence on assimilation and production rates.

Conclusions:

Our results suggest both compound-specific and ecosystem specific algal response to TOC exposure. Interestingly, while changes in community structure did occur among treatments, these changes did not correspond to changes in function. Changes in algal genera may affect feeding strategies and overall community composition of higher trophic levels, resulting in possible changes in ecosystem function. As exposure to TOCs has been documented nationally and globally, understanding the effects of this issue on all trophic levels is vital to understand effective mitigation strategies for ecosystems. Future work characterizing how response to experimental TOC exposure is coupled to historic exposure may yield insights into both TOC effects at an ecosystem scale, and possible uses of algae as indicators of TOC exposure and effects.

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Table 1. The use, chemical structure, treatment concentrations and mean concentration of compounds tested at the study site

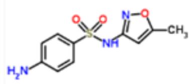
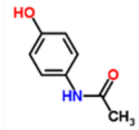
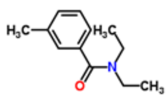
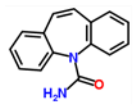
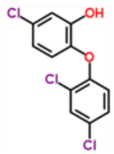
Compound	Chemical Structure	Primary Use	Treatment (ng/L)	Mean (ng/L)
Sulfamethoxazole		Human Antibiotic	2,500	286
Acetaminophen		Analgesic	10,000	24
DEET		Insect Repellant	2,500	25
Carbamazepine		Anticonvulsant	2,500	24
Triclosan		Disinfectant	10,000	< 60

Table 2. Stream characteristics of study site during 19-day incubation period of PhDS. Samples were taken at the beginning and end of the experiment, except for depth and width which were only measured once.

Characteristics	Mean
Nutrients (mg/L):	
F^-	0.08
Cl^-	46.9
NO_3^-	2.92
PO_4^{3-}	Not detected
SO_4^{2-}	81.6
Na^+	80.4
NH_4^+	Not detected
K^+	Not detected
Mg^{2+}	34.7
Ca^{2+}	84.2
DO	15.1 (mg/L)
pH	8.33
Temperature	2.55 °C
Depth	0.31 (m)
Width	20.5 (m)

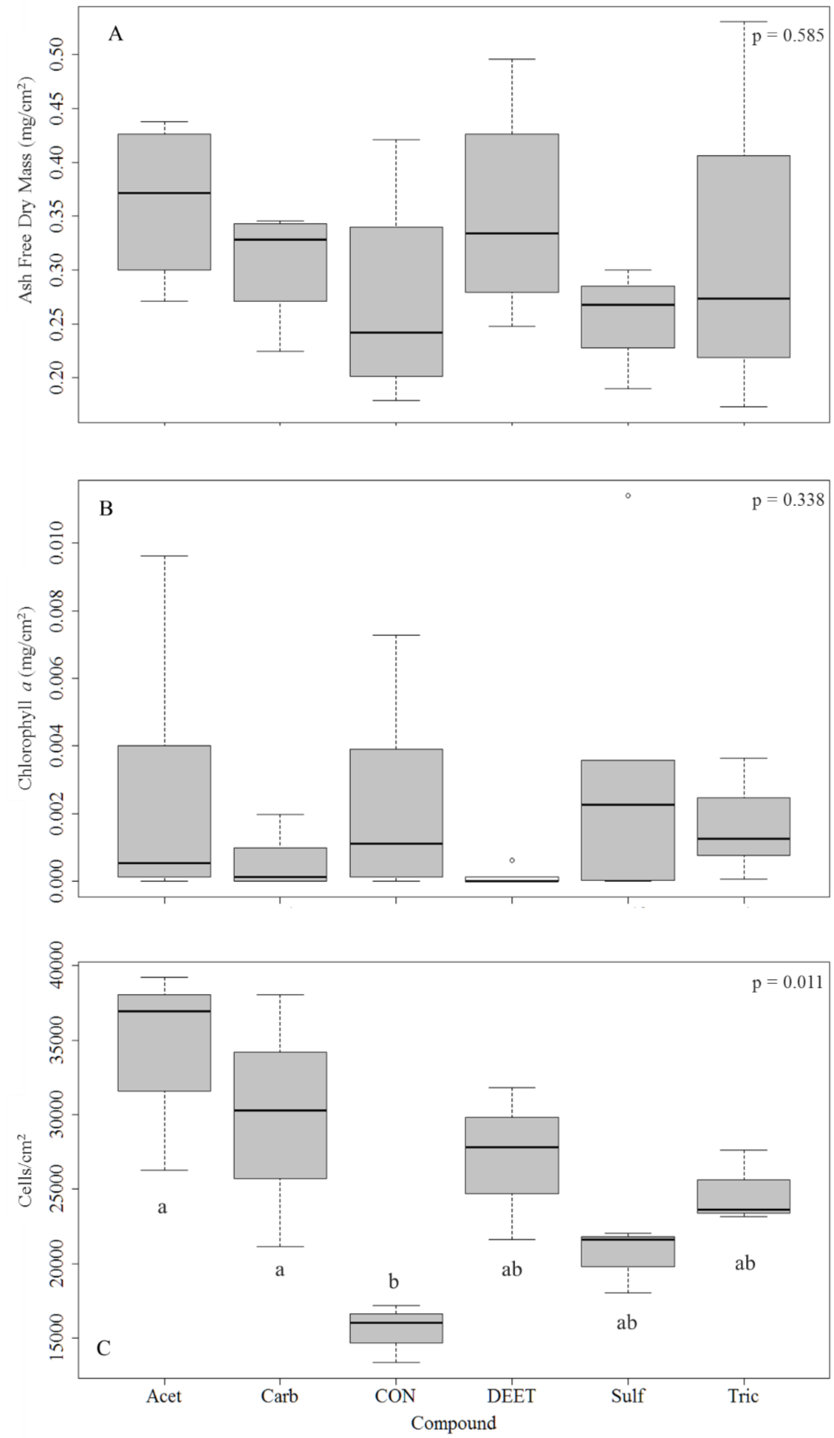


Figure 1. Growth of algal communities on fritted discs exposed to concentrations of each compound expressed as A) Ash Free Dry Mass, B) chlorophyll *a* and C) cell density. Treatments were different from one another (C) if they are not labeled with the same letter. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

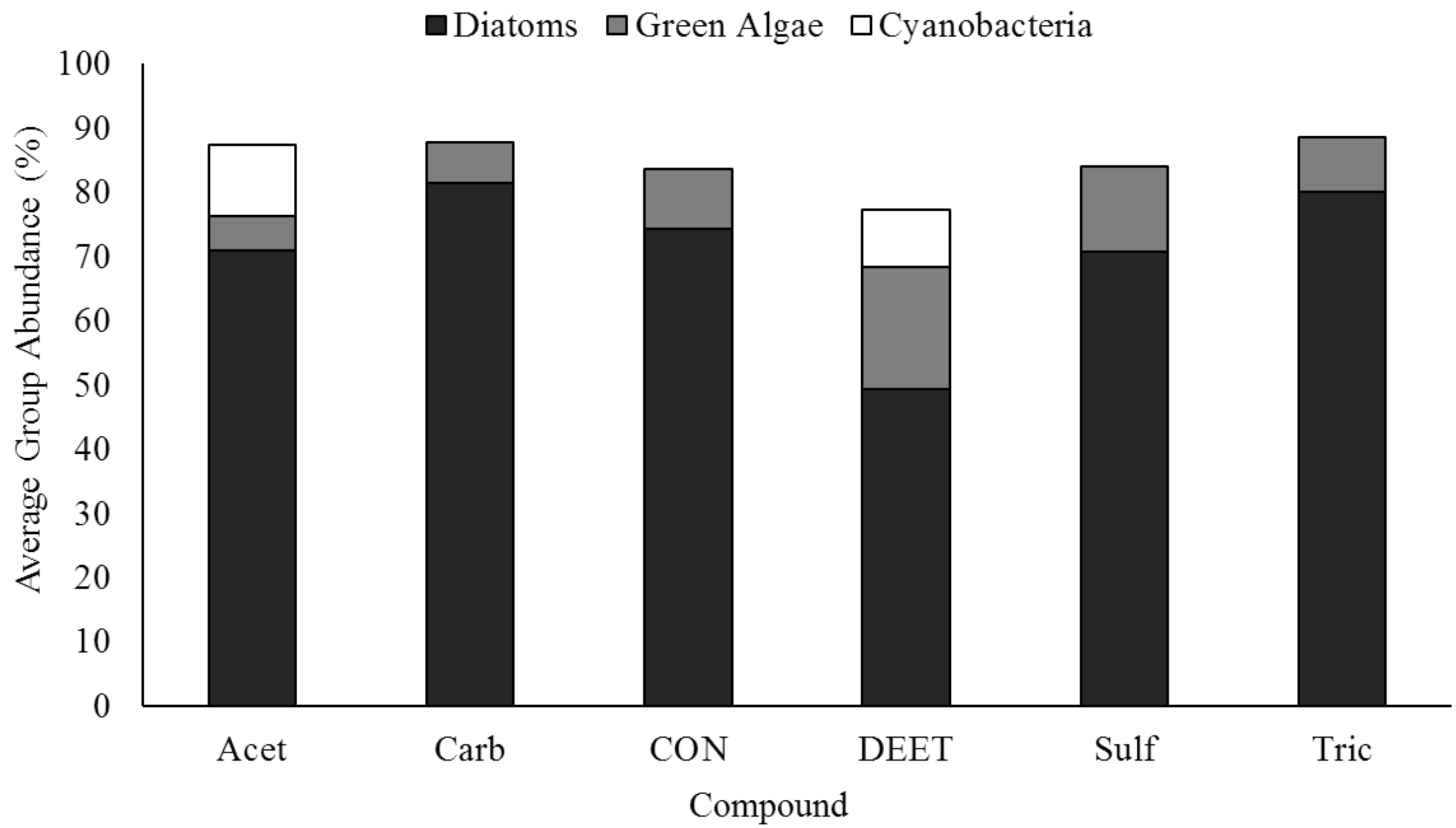


Figure 2. Average genera abundance of major algal groups grown on the fritted discs for each compound treatment. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

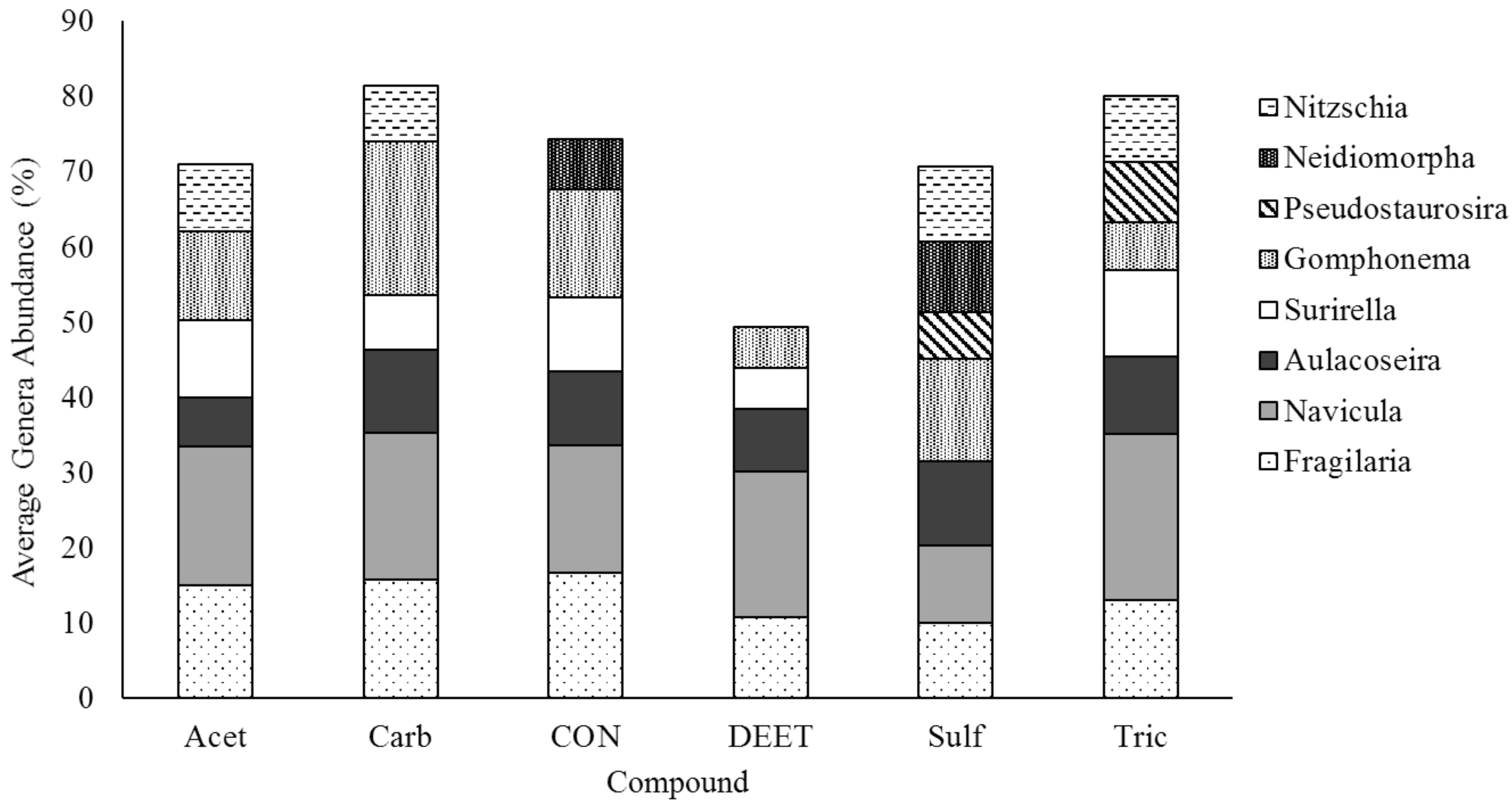


Figure 3. Diatom average genera abundance in algal communities grown on the fritted discs exposed to each treatment. Abundance was averaged across the three samples, and only genera that had an average abundance of over 5% were displayed visually. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

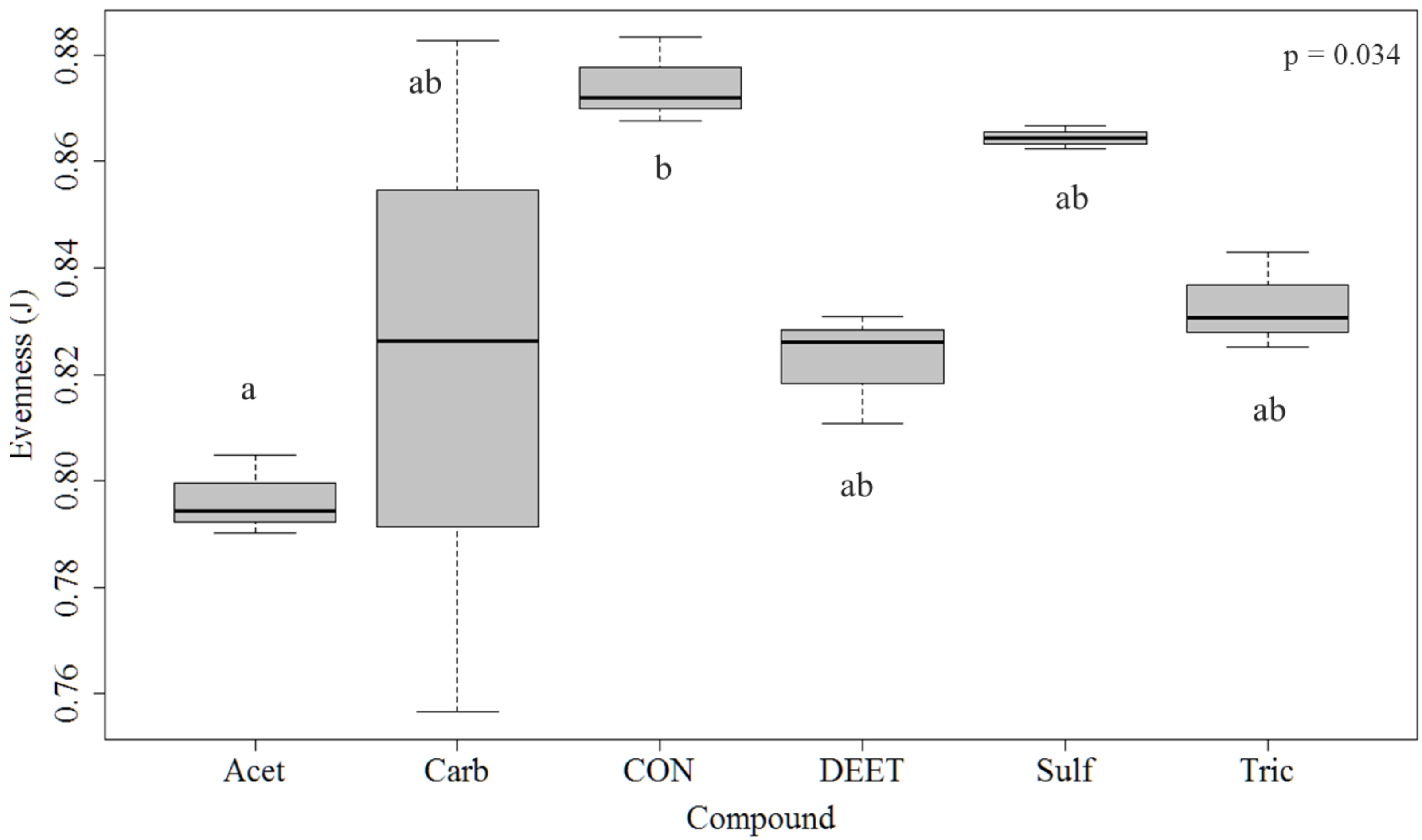


Figure 4. Evenness of the algal communities grown on the fritted discs exposed to each treatment. Treatments are different from one another if they are not labeled with the same letter. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

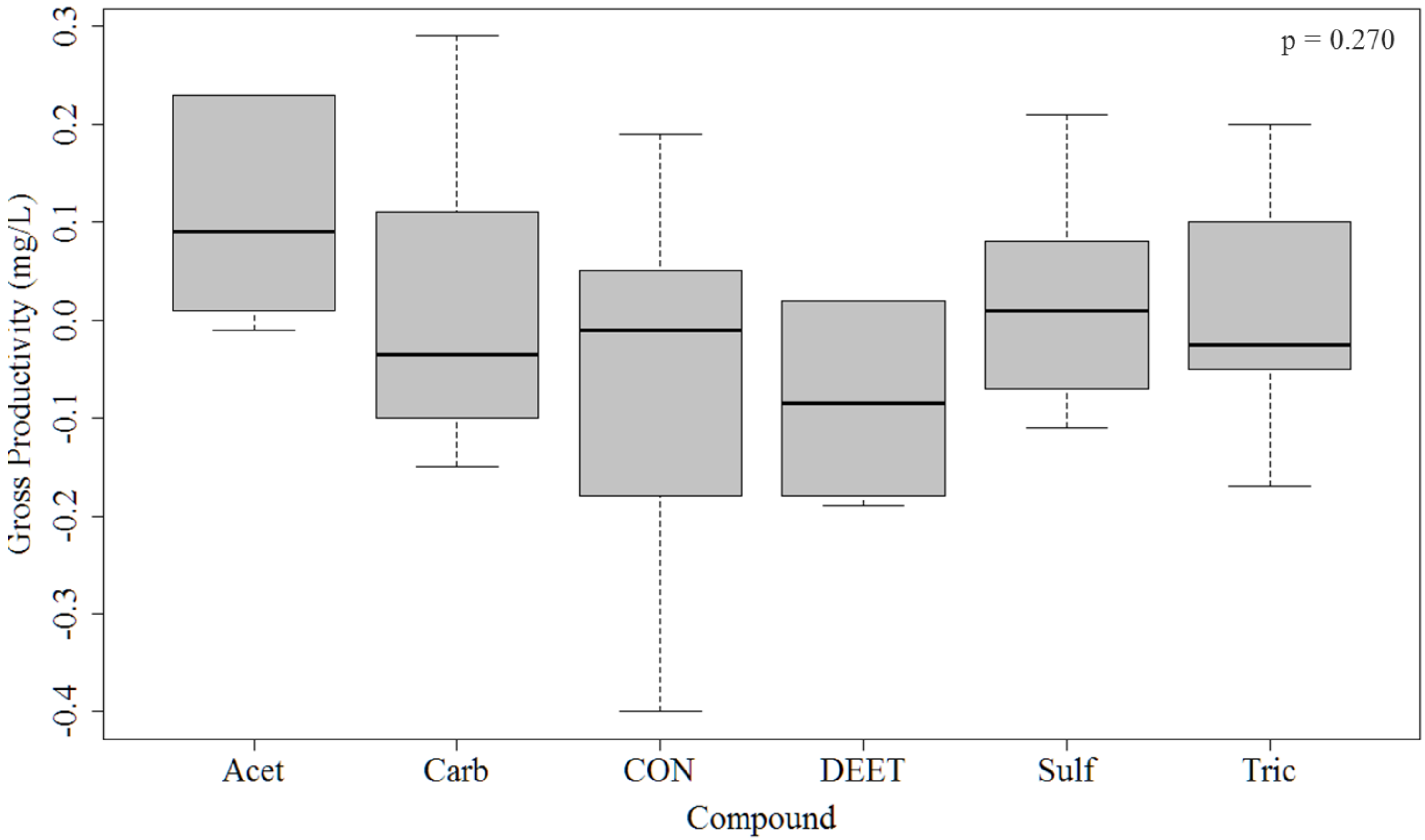


Figure 5. Gross Primary Productivity of algal communities on fritted discs placed into biological oxygen demand bottles exposed to each treatment. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

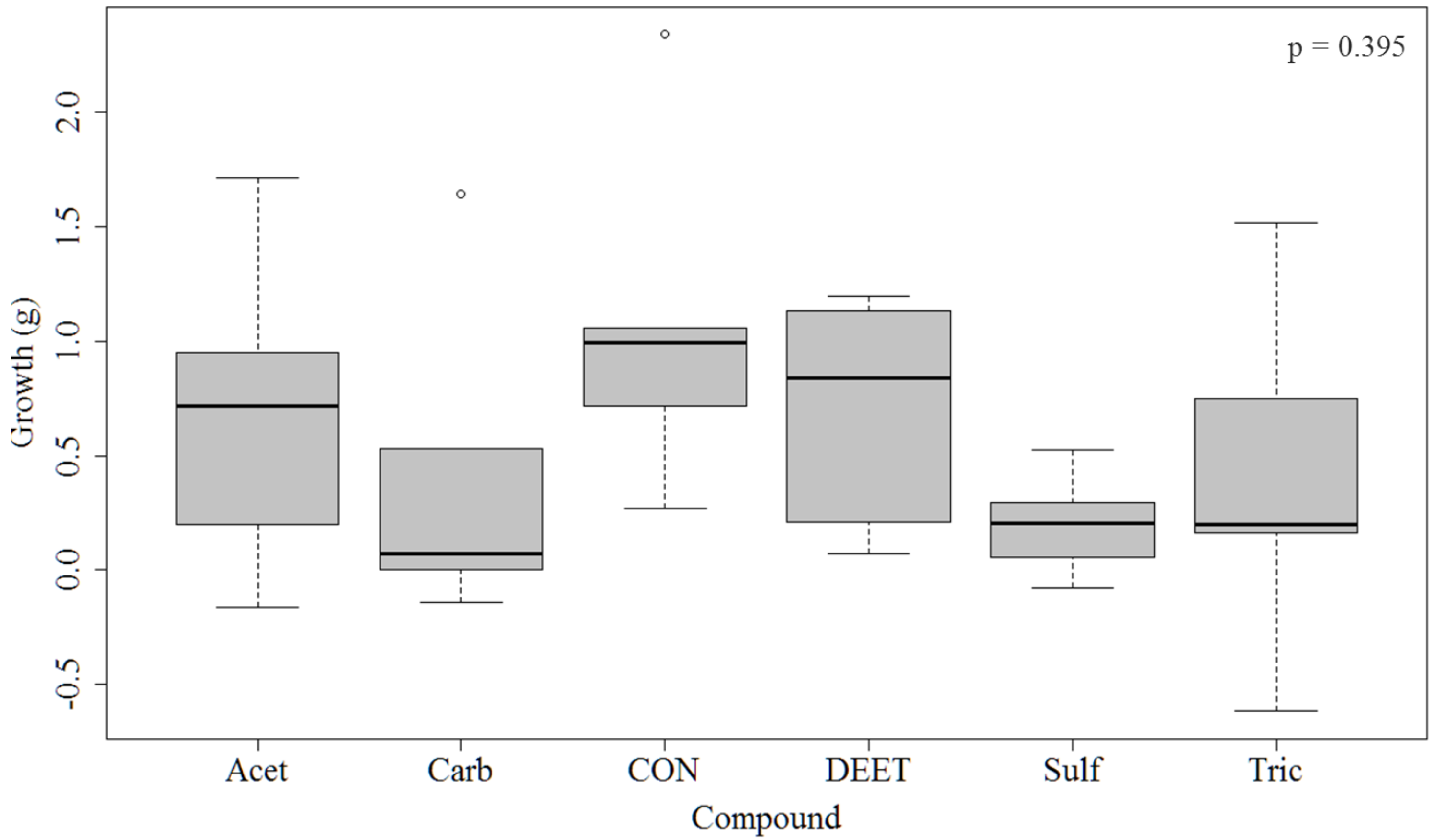


Figure 6. Change in biomass over the course of the nine-day mesocosm incubation period. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

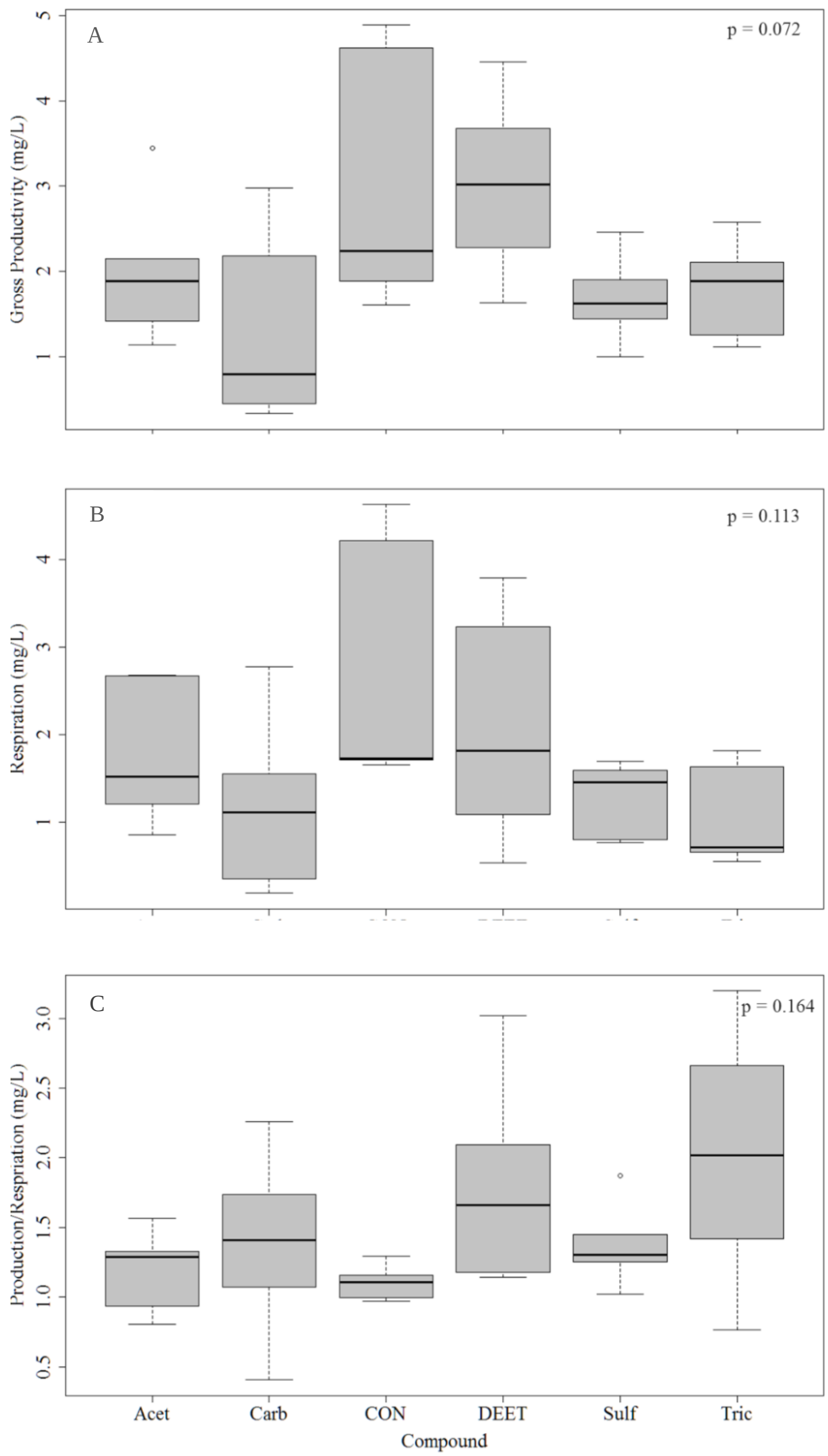


Figure 7. A) Gross primary productivity (P), B) Respiration (R) and C) a P/R ratio of algae from mesocosms placed into biological oxygen demand bottles. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

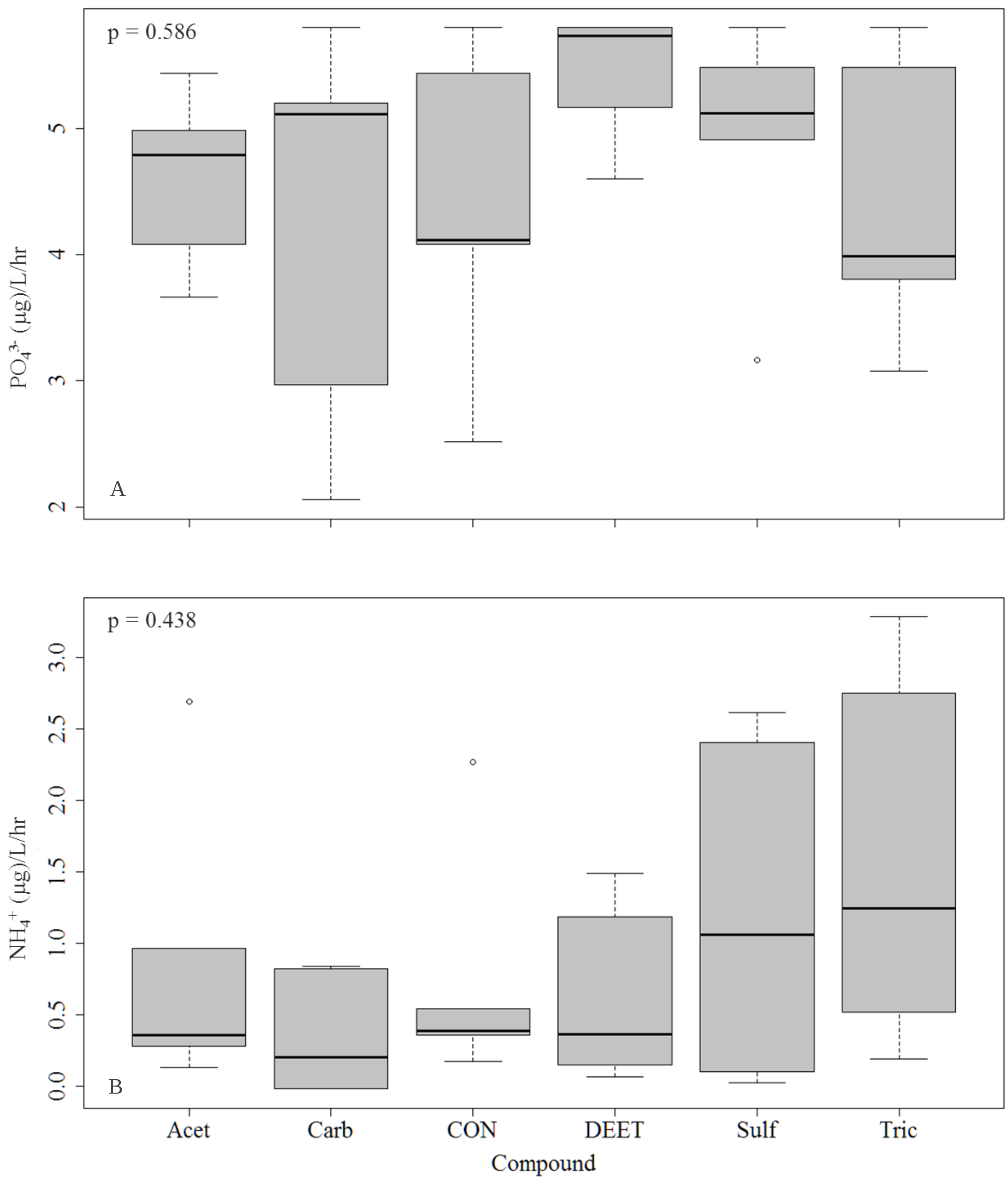


Figure 8. A) Net phosphate assimilation and B) net ammonium assimilation during the nine-day mesocosm incubation period. Acet = acetaminophen, Carb = carbamazepine, CON = control, DEET = N,N-Diethyl-meta-toluamide, Sulf = sulfamethoxazole, and Tric = triclosan.

Appendix A:

Pre-Survey Distributed to Students:

1. Approximately how often do you listen to informative or educational videos online to information?
   a. Daily
   b. Weekly
   c. Monthly
   d. Annually
   e. Never
   f. Other: (fill in)

2. Approximately how often do you read informational websites to learn information about a topic?
   a. Daily
   b. Weekly
   c. Monthly
   d. Annually
   e. Never
   f. Other: (fill in)

3. What (if any) outlets are you likely to consult for information on environmental issues? Check all that apply.
   a. Newspaper
   b. Website
   c. Online Videos
   a. TV show (News, Documentary, etc.)
   b. None
   c. Other (fill in)

4. On a scale of 1-10 (with 1 being never and 10 being all the time), how often do you consider water use in daily activities?

5. On a scale of 1-10 (with 1 being no concern and 10 being very concerned), are you concerned about water quality?

6. For the following statements, show how much you agree or disagree with each statement by clicking on one of the words below.
   a. The balance of nature is very delicate and easily upset.

STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE

   b. Humans have the right to modify the natural environment to suit their needs.

STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE

   c. All things in the natural environment are interconnected and dependent on each other.

STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE

   d. Ultimately, there's nothing individuals can do to manage or change the natural environment

STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE

7. Scenario: You or your parents bought some ibuprofen (i.e., Advil) a few years ago, and it never got used and has now expired. Your parents want to get rid of the expired medication. How will you dispose of the medication?
a. Put in the trash can
b. Pour down the drain
c. Flush down the toilet
d. Take the medication to a pharmacy
e. Take the medication to City Hall
f. Other: (fill in)

8. Scenario: It's the day after school gets out in the summer and you want to go to a friend's house about 5 blocks away. You could ask your older sibling to drive you, take your bike, walk, or use the bus that goes past your house. What form of transportation do you choose?
a. Older sibling drives you
b. Take your bike
c. Walk there
d. Take the bus
e. Other: (fill in)

9. Scenario: In order to recycle products in your house like plastic bottles, you have to buy a special kind of garbage bag. This garbage bag is $3.00 more than the normal kind you buy for regular trash. You are at the store and only have $10.00 to spend. You see bakery treats you would like to buy. If you buy the treats, you only have enough money to buy the regular bags not used for recycling. Which bags do you buy?
a. The normal bags for trash
b. The bags specifically for recycling

10. What are some negative effects of pollution on a freshwater system?

11. How much of the freshwater on Earth is drinkable?
a. 70%
b. 10%
c. 2.5%
d. 0.5%

12. Freshwater systems provide a lot of services to both humans and the organisms that live there. List 2-3 services a freshwater system can provide.

13. Water pollution is a problem in many areas of the world. Thankfully, there are several things people can do to help decrease water pollution. List 2-3 ways you could help decrease water pollution.

Appendix B:

Post Survey Distributed to Students:

1. What (if any) outlets are you likely to consult for information on environmental issues? Check all that apply.
   a. Newspaper
   b. Website
   c. Online Videos
   d. TV show (News, Documentary, etc.)
   e. None
   f. Other (fill in)

2. On a scale of 1-10 (with 1 being never and 10 being all the time), how often do you consider water use in daily activities?

3. On a scale of 1-10 (with 1 being no concern and 10 being very concerned), are you concerned about water quality?

4. For the following statements, show how much you agree or disagree with each statement by clicking on one of the words below.
   a. The balance of nature is very delicate and easily upset.
   STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE
   b. Humans have the right to modify the natural environment to suit their needs.
   STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE
   c. All things in the natural environment are interconnected and dependent on each other.
   STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE
   d. Ultimately, there's nothing individuals can do to manage or change the natural environment
   STRONGLY DISAGREE DISAGREE UNSURE AGREE STRONGLY AGREE

5. Scenario: You or your parents bought some ibuprofen (i.e., Advil) a few years ago, and it never got used and has now expired. Your parents want to get rid of the expired medication. How will you dispose of the medication?
   a. Put in the trash can
   b. Pour down the drain
   c. Flush down the toilet
   d. Take the medication to a pharmacy
   e. Take the medication to City Hall
   f. Other: (fill in)

6. Scenario: It’s the day after school gets out in the summer and you want to go to a friend’s house about 5 blocks away. You could ask your older sibling to drive you, take your bike, walk, or use the bus that goes past your house. What form of transportation do you choose?
   a. Older sibling drives you

b. Take your bike
c. Walk there
d. Take the bus
e. Other: (fill in)

7. Scenario: In order to recycle products in your house like plastic bottles, you have to buy a special kind of garbage bag. This garbage bag is $3.00 more than the normal kind you buy for regular trash. You are at the store and only have $10.00 to spend. You see bakery treats you would like to buy. If you buy the treats, you only have enough money to buy the regular bags not used for recycling. Which bags do you buy?
a. The normal bags for trash
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8. What are some negative effects of pollution on a freshwater system?

9. How much of the freshwater on Earth is drinkable?
a. 70%
b. 10%
c. 2.5%
d. 0.5%

10. Freshwater systems provide a lot of services to both humans and the organisms that live there. List 2-3 services a freshwater system can provide.

11. Water pollution is a problem in many areas of the world. Thankfully, there are several things people can do to help decrease water pollution. List 2-3 ways you could help decrease water pollution.

12. On a scale of 1-10 (with one being nothing and 10 being very much) how much do you feel you learned from this activity?

13. On a scale of 1-10(with 1 being very unclear and 10 being very clear) how clearly do you think the material was presented to you?

14. On a scale of 1-10 (with 1 being not effective at all and 10 being very effective) how effective do you think the activity was at helping you learn information?

15. Do you have any suggestions for ways the activity could be improved to help you learn better?

Appendix C:

2nd Post Survey Distributed to Students:

1. What are some negative effects of pollution on a freshwater system?

2. How much of the freshwater on Earth is drinkable?
e. 70%
f. 10%
g. 2.5%
h. 0.5%

3. Freshwater systems provide a lot of services to both humans and the organisms that live there. List 2-3 services a freshwater system can provide.

4. Water pollution is a problem in many areas of the world. Thankfully, there are several things people can do to help decrease water pollution. List 2-3 ways you could help decrease water pollution.

Appendix D:

Pamphlet Communication Mode:

How Do Humans Influence Water Quality?

Why Do We Care About Water?

Water is one of the most important resources on our planet because it is finite, meaning we cannot create more. About 70% of the Earth is covered in water; however, only 2.5% of that water is freshwater, and breaking that down even further, only 0.5% of that water is drinkable. Human beings themselves are made up of about 60% water and therefore need water daily to perform necessary functions in our bodies like moving around important nutrients[1]. Having clean water is important to make sure our bodies stay healthy, and aren't harmed by bacteria, viruses, heavy metals, or other pollutants that can be in water.

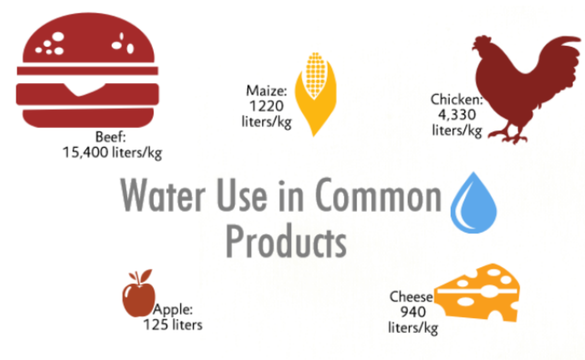
Freshwater also provides a lot of ecosystem services. Ecosystem services are ways an ecosystem—a group of organisms and their environment—fill a role or benefit another group[2]. For example, freshwater is used for

- Cleaning
- Growing food such as agriculture crops or fish
- Generating electricity
- Recreation and tourism[2]

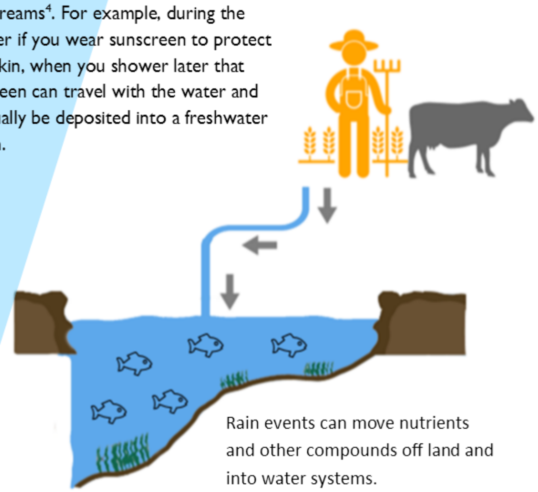
Freshwater also supports thousands of organisms and allows diverse communities to exist. For example, a river running through a prairie can provide a spot for plants to grow and aquatic animals to feed the land animals.

Freshwater Pollution: How Does It Get There?

Pollution can have a big impact on the quality of freshwater and how much is available to be used for different ecosystem services. For example, water is used in the production of most of our food and products that human beings use. The amount of water it takes to make just one 1/3 pound hamburger is 2,310 liters. To put that into perspective, the average shower (8.2 minutes) uses 65 liters of water[3]. A lot of this water does not make it back into water's cyclical journey on Earth called The Water Cycle, and therefore can't be used again.



After falling down from the sky as Precipitation, water can pick up pollutants on its way back into freshwater. When water goes down the drain in our homes and buildings it generally flows through a wastewater treatment plant. This plant uses several techniques to treat the water and make it safe to go back into freshwater ways like streams and rivers. However, these treatments aren't always 100% effective and often compounds that you use every day are introduced back into streams[4]. For example, during the summer if you wear sunscreen to protect your skin, when you shower later that sunscreen can travel with the water and eventually be deposited into a freshwater system.



Rain events can move nutrients and other compounds off land and into water systems.

Pollutants can also move into freshwater systems by being carried with rain water. During a rain event, water can pick up compounds and move them into a river or stream where it can have an effect on aquatic life[5]. For example, farming uses fertilizers like nitrogen and phosphorus. While these elements occur naturally and are used by aquatic organisms, excess nutrients from manure and other applications can be moved into rivers and streams with rainfall.

How Does Pollution Affect Water?

Several pollutants are found in freshwater. Below are some examples of common pollutants and their effects on aquatic ecosystems:

- **Nutrients:** High levels of nutrients like phosphorus and nitrogen can change ecosystem structure. These changes can lead to harmful effects like algal blooms, in which algae grow in large amounts depleting oxygen for other organisms. Large algal blooms can also produce toxins that can be harmful to aquatic life and even other animals like dogs and humans[6]. When these blooms happen in lakes used for recreation, it can often shut down beaches and other areas leading to very costly removal techniques.

Photo Credit: Jeremy Walls

Posted at Grand Lake St. Mary's in Ohio during summer 2015

- **Common Use Products:** Lots of products we use every day like hand soaps, shampoos, prescription drugs, sunscreens, antibiotics, and insect repellants are found in freshwater systems[4]. Often, the ingredients in these products are not made to stay in or on the human body. As a result, compounds can get into freshwater systems through wastewater after people use them. They can also enter systems through industrial processes like farming or manufacturing[5]. For example, if cows are treated with antibiotics, those antibiotics can make it into freshwater after manure is applied to a field. Also, a factory that manufactures antibiotics could introduce antibiotics into a river or stream through its wastewater.

The effects of common use products on aquatic organisms are still being studied. Research looking at this issue shows some compounds can affect organism function. Fish are shown to have behavior changes, as well as body changes, that can impact how successful they are at reproducing[8]. If fish are not able to reproduce or survive as well, their population numbers could go down. Decreasing fish populations could have negative impacts on how much fish is available to eat, and how an ecosystem is structured.

Some common examples of products we use that contain compounds found in freshwater.

How Can We Help?

There are a lot of simple things we can do to help reduce pollution, improve water quality, and promote ecosystem heath. We can especially make a difference in reducing the amount of products we use every day:

- Do not flush pills, chemicals, or other medications down the drain
- Clean up after your pets
- Don't buy more than you need, this will reduce the amount disposed.
- Only take the recommended dosage for the recommended amount of time. If you take extra, that can put more compounds in the environment
- Ask your pharmacy if you can dispose of unused drugs.
- See if your local city hall has a drug take-back day where you can dispose of unused drugs in an environmentally safe way

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(3) Water Footprint Network Water Use Assessment Tool. 2016. Web. http://waterfootprint.org.
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(8) Niemuth, J and R. Klaper. 2015. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. Environ Sci Technol 135: 38-45.

Authors: Brittany A. Maule, Melody J. Bernot
Biology Department, Ball State University
bmaule@bsu.edu
mjbernot@bsu.edu

BALL STATE UNIVERSITY

Appendix E:

Website Communication Mode:

http://ppcpedproject.weebly.com/

Appendix F:

Video Communication Mode:

https://drive.google.com/file/d/0B9PScfFgrlmALVl5enVENEYycDQ/view?usp=sharing